Evaluation Report
proficiency test

DLA ptAL09 (2021)

Allergens IX:

Peanut and Almond

in Barbecue Spice Mixture

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

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EP-Nummer *PT-Number*	DLA ptAL09 (2021)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (1 February 2022) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 1 February 2022
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
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.
Akkreditierung *Accreditation* ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-EP-21534-01-00	nach / according to: ISO/IEC 17.043-2010 Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen Conformity Assessment - General Requirements for Proficiency Testing Die Akkreditierung gilt für den in der Urkundenanlage genannten Umfang. The accreditation is valid for the scope of the annex to the certificate of accreditation

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1. Introduction

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

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 food matrix sample test material is a barbecue spice mixture prepared by DLA from commercially available paprika, garlic and onion powders. The basic composition was the same for both samples A and B (see Table 1).
After sieving (mesh <600 µm), the basic mixture was homogenized.
The **spiked sample B** was then prepared as follows:
The spiking materials containing the allergenic ingredients peanut and almond were added to an aliquot of the base matrix and the mixture was homogenized. Base matrix was then added in portions again and homogenized in each case until the total amount was reached.
The **spiking level sample** was produced with the above-mentioned allergen-containing spiking materials with multi-stage addition of potato powder (mesh 500 µm) and homogenization.

Samples A and B were filled in portions of approx. 25 g and the spiking level sample of approx. 15 g in metallized PET film bags.

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Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Barbecue Spice Mixture Ingredients: paprika powder (47%), garlic powder (26%), onion powder (27%)	100 g/100 g	99,9 g/100g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,9 g/100g
Peanut, roasted milled, mixture (18 products from USA, Asia, Africa, South America) - as peanut* - *thereof 23,2% total protein***	-	 25,7 mg/kg 5,96 mg/kg	 15,8 mg/kg 3,66 mg/kg
Almond, roasted milled, mixture (23 products from USA, Europe, Australia, Western Asia) - as almond* - *thereof 21,1% total protein***	-	 26,3 mg/kg 5,55 mg/kg	 19,3 mg/kg 4,08 mg/kg
Further Ingredients: *Maltodextrin, sodium sulfate and silicon dioxide*	-	<0,1 g/100 g	<0,1 g/100 g

* Allergen contents as "total food" as described in the column ingredients according to the gravimetric mixture
** Protein contents according to laboratory analysis of the raw material (total nitrogen according to Kjeldahl with F=5,46 for peanut protein and F=5,18 for almond protein)

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

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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 60% and 95%. 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]. HorRat values of 1,1 and 0,75 were obtained in this PT. 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 (exception: Morinaga ELISA II performed by DLA). 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, Morinaga and AgraQuant) and almond (Immunolab, Veratox and AgraQuant) (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].

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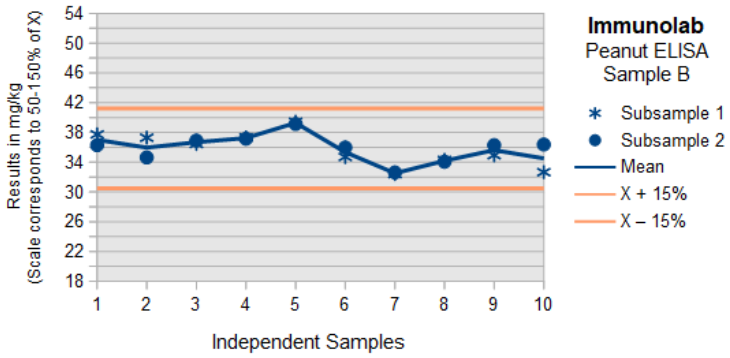
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 35,8 ± 1,8 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	37,7	36,3	37,0
2	37,3	34,6	36,0
3	36,5	36,9	36,7
4	37,3	37,2	37,2
5	39,4	39,1	39,3
6	34,7	36,0	35,3
7	32,4	32,6	32,5
8	34,3	34,1	34,2
9	34,9	36,3	35,6
10	32,7	36,4	34,5

General average X	35,8	
SD of sample means Sx	1,88	5,2%
SD within-samples Sw	0,82	2,3%
SD between-samples Ss	1,79	5,0%

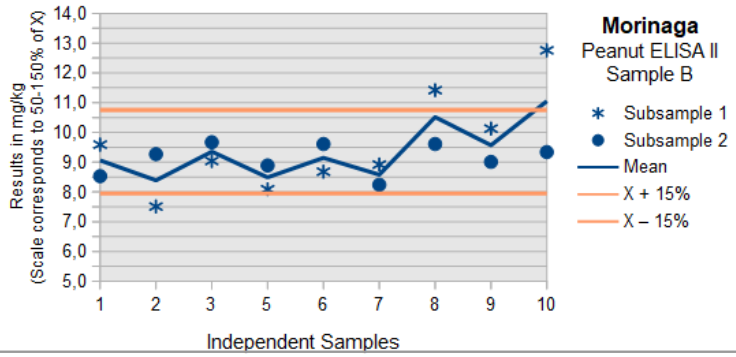


Morinaga Peanut ELISA Kit II (High Sensitive Kit II)

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut Protein 9,35 ± 0,72 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	9,59	8,53	9,06
2	7,51	9,27	8,39
3	9,04	9,67	9,36
5	8,09	8,89	8,49
6	8,68	9,61	9,15
7	8,92	8,24	8,58
8	11,4	9,61	10,5
9	10,1	9,01	9,57
10	12,8	9,34	11,05

General average X	9,35	
SD of sample means Sx	0,91	9,8%
SD within-samples Sw	0,80	8,5%
SD between-samples Ss	0,72	7,7%

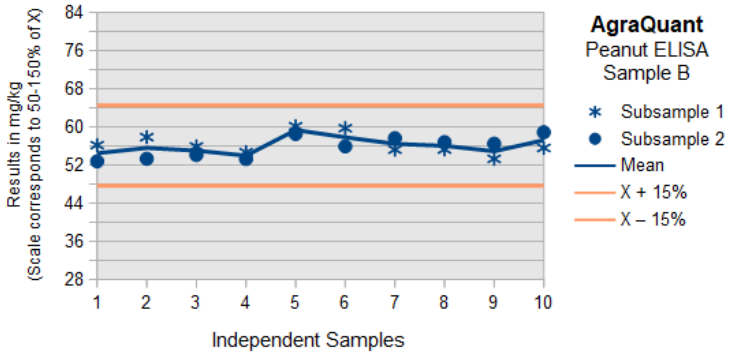


AgraQuant Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 56,1 ± 1,3 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	56,1	52,8	54,4
2	57,8	53,3	55,6
3	55,9	54,1	55,0
4	54,7	53,2	54,0
5	60,1	58,5	59,3
6	59,7	55,9	57,8
7	55,2	57,6	56,4
8	55,2	56,8	56,0
9	53,3	56,5	54,9
10	55,6	58,8	57,2

General average X	56,1	
SD of sample means Sx	1,66	3,0%
SD within-samples Sw	1,45	2,6%
SD between-samples Ss	1,31	2,3%



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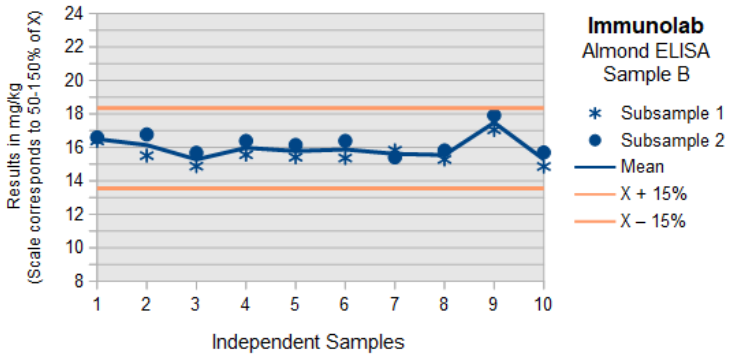
ELISA-Tests: Homogenität Mandel / Homogeneity Almond

Immunolab Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 15,9 ± 0,59 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	16,4	16,6	16,5
2	15,5	16,8	16,1
3	14,9	15,7	15,3
4	15,6	16,4	16,0
5	15,4	16,1	15,8
6	15,4	16,4	15,9
7	15,8	15,4	15,6
8	15,3	15,8	15,5
9	17,0	17,9	17,5
10	14,9	15,7	15,3

General average X	15,9	
SD of sample means Sx	0,66	4,1%
SD within-samples Sw	0,40	2,5%
SD between-samples Ss	0,59	3,7%

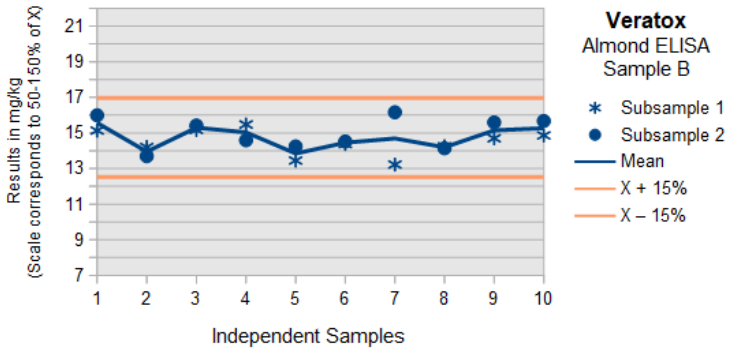


Veratox Almond ELISA

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Almond 14,7 ± 0,46 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	15,1	16,0	15,6
2	14,2	13,7	14,0
3	15,2	15,4	15,3
4	15,5	14,6	15,0
5	13,4	14,2	13,8
6	14,4	14,5	14,4
7	13,2	16,2	14,7
8	14,3	14,1	14,2
9	14,7	15,6	15,1
10	14,9	15,7	15,3

General average X	14,7	
SD of sample means Sx	0,61	4,1%
SD within-samples Sw	0,56	3,8%
SD between-samples Ss	0,46	3,1%

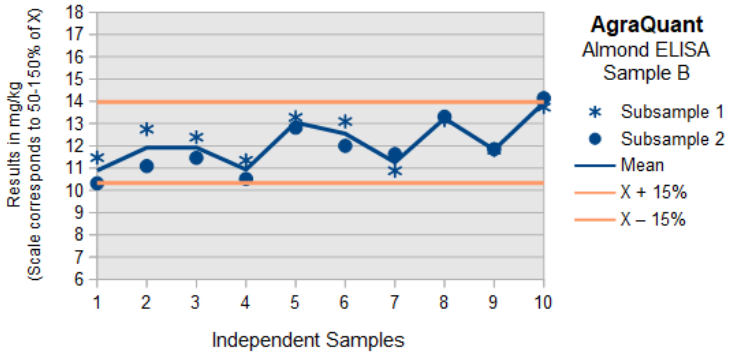


AgraQuant Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 12,2 ± 0,97 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	11,5	10,3	10,9
2	12,7	11,1	11,9
3	12,4	11,5	11,9
4	11,4	10,5	10,9
5	13,3	12,8	13,0
6	13,1	12,0	12,5
7	10,9	11,6	11,3
8	13,2	13,3	13,2
9	11,8	11,9	11,8
10	13,7	14,1	13,9

General average X	12,2	
SD of sample means Sx	1,02	8,4%
SD within-samples Sw	0,44	3,6%
SD between-samples Ss	0,97	8,0%



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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 sample B was approx. 0,42 (20,6°C) and of the spiking level sample about 0,50 (19,1°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 37th week of 2021. The testing method was optional. The tests should be finished at 12th November 2021 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 Almond in the range of mg/kg in the matrix of Barbecue Spice Mixture. 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 11 participants submitted at least one result.

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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].

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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^*_{ALL}***
ii) ***Robust standard deviation of single methods - $S^*_{METHOD\ i}$***
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]. Also, 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. 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.

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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}.

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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	σpt	Method / Literature
Peanut	Milk chocolate	173,7 33,8 5,9	87 % 85 % 59 %	- - -	8,8% 5,2% 7,8%	31% 20% 31%	30,4% 19,7% 30,5%	ELISA Manuf. A ASU 00.00-69
Peanut	Milk chocolate	215,7 40,1 10,1	108 % 100 % 101 %	- - -	5,9% 7,2% 7,3%	32% 14% 16%	31,7% 13,0% 15,1%	ELISA Manuf. B ASU 00.00-69
Peanut	Dark chocolate	148,2 30,9 5,7	74 % 77 % 57 %	- - -	6,0% 13% 6,1%	22% 25% 33%	21,6% 23,2% 32,7%	ELISA Manuf. A ASU 00.00-69
Hazelnut	Dark chocolate	16,3 7,56 3,73 1,62	81 % 76 % 75 % 81 %	- - - -	4,7% 8,9% 13% 15%	12% 15% 24% 33%	11,5% 13,6% 22,2% 31,2%	ELISA Manuf. A ASU 44.00-7
Hazelnut	Dark chocolate	21,3 10,7 4,69 2,37	106 % 107 % 94 % 119 %	- - - -	7,1% 11% 11% 9,3%	14% 19% 17% 17%	13,1% 17,3% 15,1% 16,4%	ELISA Manuf. B ASU 44.00-7

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 12 – 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%.

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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} [32-35]

Parameter	Matrix	Mean [mg/kg]	Recov-ery	rob RSD	RSD_r	RSD_R	σ_{pt}	Method / Literature
Peanut	Rice biscuits	23,4 5,19	113 % 99,7 %	15,6% 15,0%	11,6% 14,7%	14,4% 18,1%	11,8% 14,8%	rt-PCR ASU 00.00-169
Peanut	Wheat biscuits (DLA)	1,97	39,3 %	16,2%	16,0%	19,5%	15,8%	rt-PCR ASU 00.00-169
Peanut	Milk powder Boiled sausage	3,66 2,44	73,2 % 49,4 %	15,8% 15,6%	12,8% 11,9%	14,8% 15,9%	11,7% 13,5%	rt-PCR ASU 00.00-169
Almond	Rice biscuits	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 biscuits 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 biscuits	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 biscuits 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 biscuits	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 biscuits 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 biscuits	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 biscuits 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

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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.

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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 evaluation 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].

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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.

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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 participants' 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.
The corresponding z-scores were calculated according to 3.5 with the target standard deviation of 25% (see 3.4.3).

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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 (valid digits). 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.
An ELISA result was given as **peanut protein** and converted to the **total food (peanut)** using the experimentally determined protein content of the raw materials (see page 5).
The ELISA results for the parameter almond were given consistently as whole food, so that no conversions were necessary.

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.

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

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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.

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4.1 Proficiency Test Peanut

4.1.1 ELISA Results: Peanut

Qualitative evaluation of the 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	<1	positive	23,4	1/1 (100%)	BF	
10	negative	<LOQ	positive	40,4	1/1 (100%)	BF	
1	positive	1,10	positive	28,4	1/1 (100%)	BK	
9	positive	1,00	positive	22,0	1/1 (100%)	BK	
3	positive	1,64	positive	32,8	1/1 (100%)	MI-II	result converted °
4	positive	1,90	positive	34,6	1/1 (100%)	RS	
6	positive	1,81	positive	35,8	1/1 (100%)	RS	
7	positive		positive		1/1 (100%)	RS	
8	positive	1,97	positive	>6,00	1/1 (100%)	RS	
5	negative	<2,5	positive	24,7	1/1 (100%)	RS-F	
11	positive	1,80	positive	37,0	1/1 (100%)	SP	

° calculation see p. 19

	Sample A		Sample B	
Number positive	8		11	
Number negative	3		0	
Percent positive	73		100	
Percent negative	27		0	
Consensus value	no		positive	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comments:

The positive consensus value for sample B is in qualitative agreement with the spiking of sample B. 3 negative results were given for sample A, so that a consensus value of ≥ 75% could not be determined. The positive results for sample A were all in the range of 1-2 mg/kg.

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Quantitative evaluation of ELISA-results: Sample A

Evaluation number	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	<1		BF	
10	<LOQ		BF	
1	1,10	-1,6	BK	
9	1,00	-1,8	BK	
3	1,64	-0,36	MI-II	result converted °
4	1,90	0,22	RS	
6	1,81	0,02	RS	
7			RS	
8	1,97	0,38	RS	
5	<2,5		RS-F	
11	1,80	0,00	SP	

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comment:
A kernel density estimation was not made due to the number of < 8 results.

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Characteristics: Quantitative evaluation ELISA Peanut

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	7
Number of outliers	0
Mean	1,60
Robust Mean	1,60
Median (X_{pt})	**1,80**
Robust standard deviation (S*)	**0,445**
Target range:	
Target standard deviation σ_{pt}	**0,450**
lower limit of target range	**0,900**
upper limit of target range	**2,70**
Quotient S/σ_{pt}*	*0,99*
Standard uncertainty $U_{(Xpt)}$	*0,210*
Results in the target range	*7*
Percent in the target range	*100*

Assigned value (Xpt): Median (see 3.1)

Comments to the statistical characteristics and assigned values:

The evaluation of the results of all methods showed a low variability of the results. The quotient S/σpt was below 1,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (cf. 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.*

Peanut was not added to sample A, therefore no recovery rate can be calculated.

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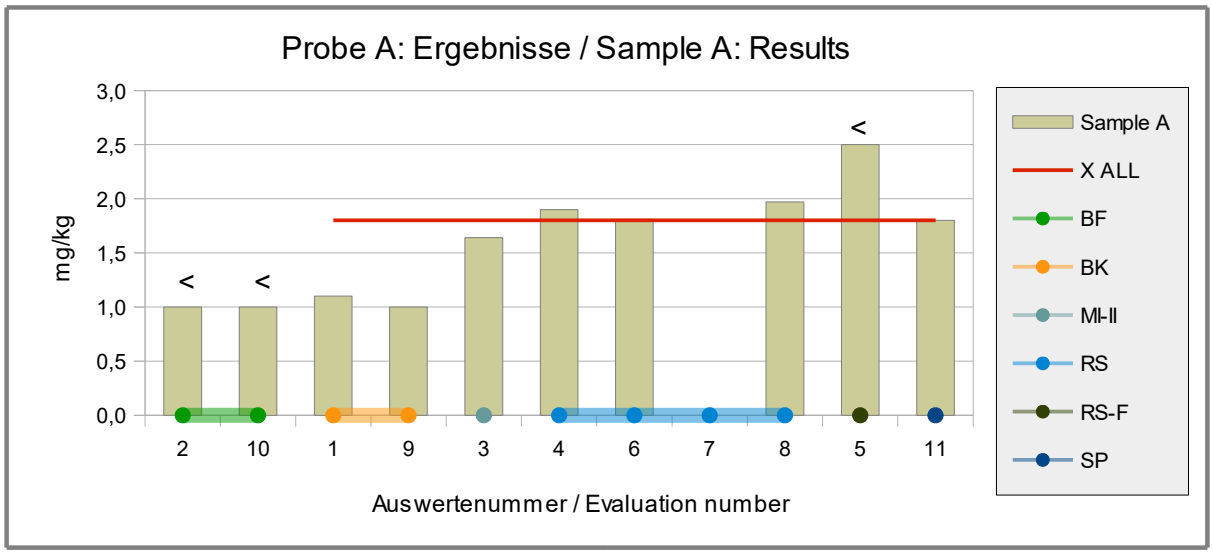

Abb./Fig. 1: ELISA Results Peanut
red line = Assigned value median of all methods
round symbols = Applied methods (see legend)

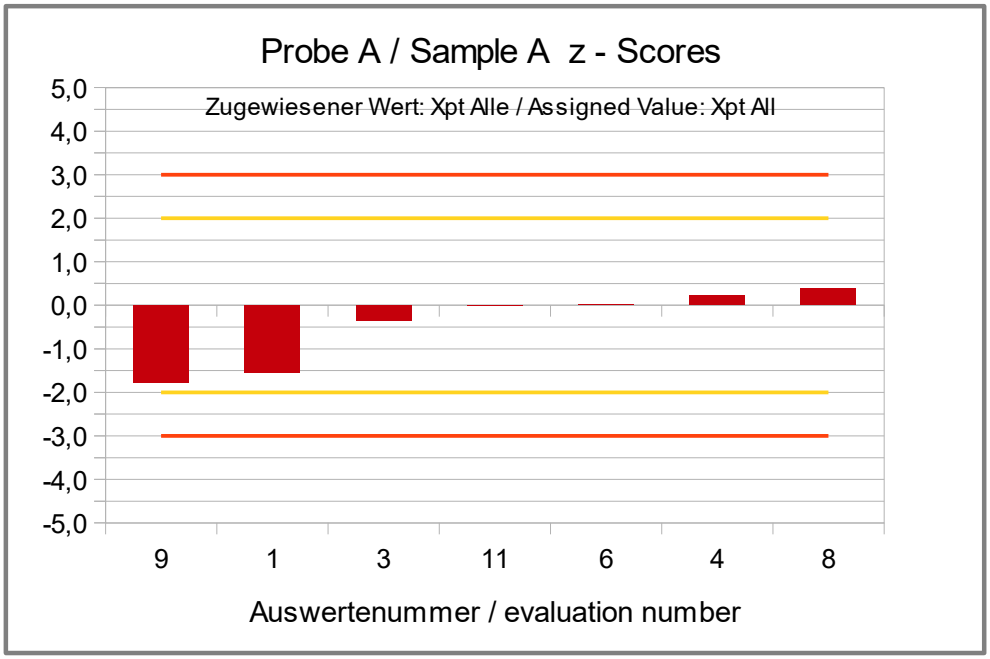

Abb./Fig. 2:
z-Scores (ELISA Results Peanut)
Assigned value median of all results

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Quantitative evaluation of ELISA-results: Sample B

Evaluation number	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	23,4	-0,98	BF	
10	40,4	1,2	BF	
1	28,4	-0,33	BK	
9	22,0	-1,2	BK	
3	32,8	0,23	MI-II	result converted °
4	34,6	0,46	RS	
6	35,8	0,62	RS	
7			RS	
8	>6,00		RS	
5	24,7	-0,81	RS-F	
11	37,0	0,77	SP	

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

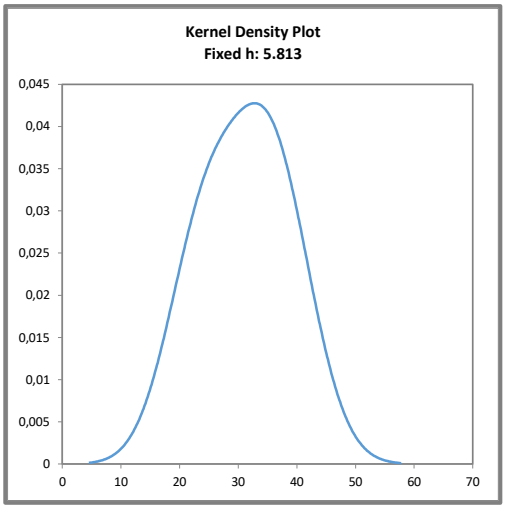

Abb. / Fig. 3:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an almost symmetrical distribution of the results.

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Characteristics: Quantitative evaluation ELISA Peanut

Sample B

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	9
Number of outliers	0
Mean	31,0
Median	32,8
Robust Mean (X_{pt})	**31,0**
Robust standard deviation (S*)	**7,48**
Target range:	
Target standard deviation σ_{pt}	**7,75**
lower limit of target range	**15,5**
upper limit of target range	**46,5**
Quotient S/σ_{pt}*	*0,96*
Standard uncertainty $U_{(Xpt)}$	*3,12*
Results in the target range	*9*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The kernel density showed almost a symmetrical distribution.

The evaluation of the results of all methods showed a low variability of the results. The quotient S/σpt was below 1,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (cf. 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 mean of the evaluation was 121% of the spiking level of peanut to sample B and thus within the range of the recommendations for the applied methods (see 3.4.3 and p. 31 "Recovery rates with z-scores ELISA for Peanut"). The fact that the basic matrix contains small amounts of peanuts in the range of 1-2 mg/kg (see results of sample A) was not taken into account here.

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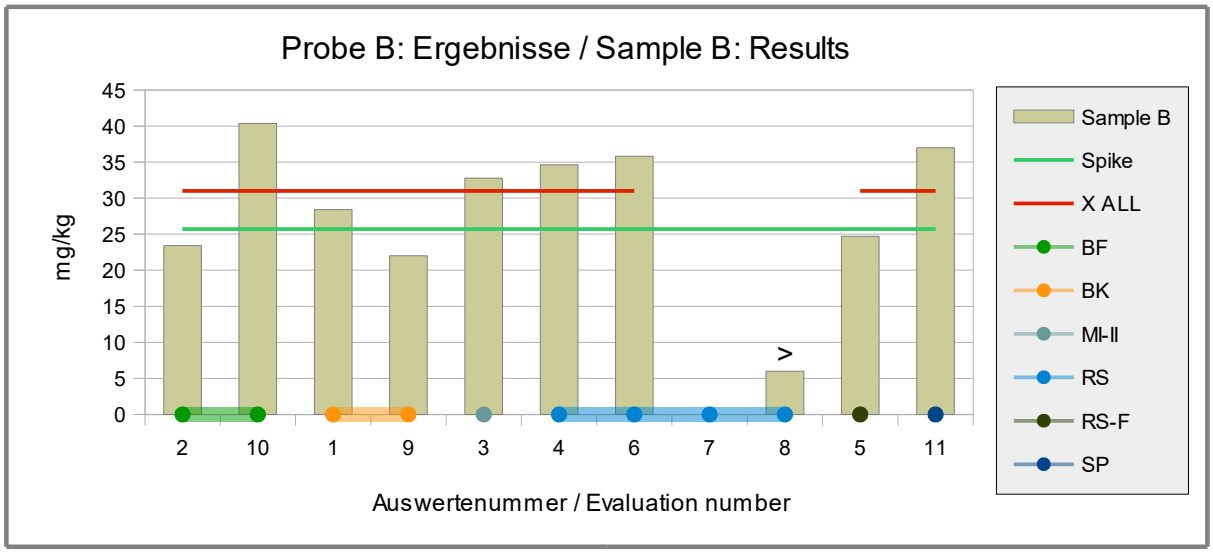

Abb./Fig. 4: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all methods
round symbols = Applied methods (see legend)

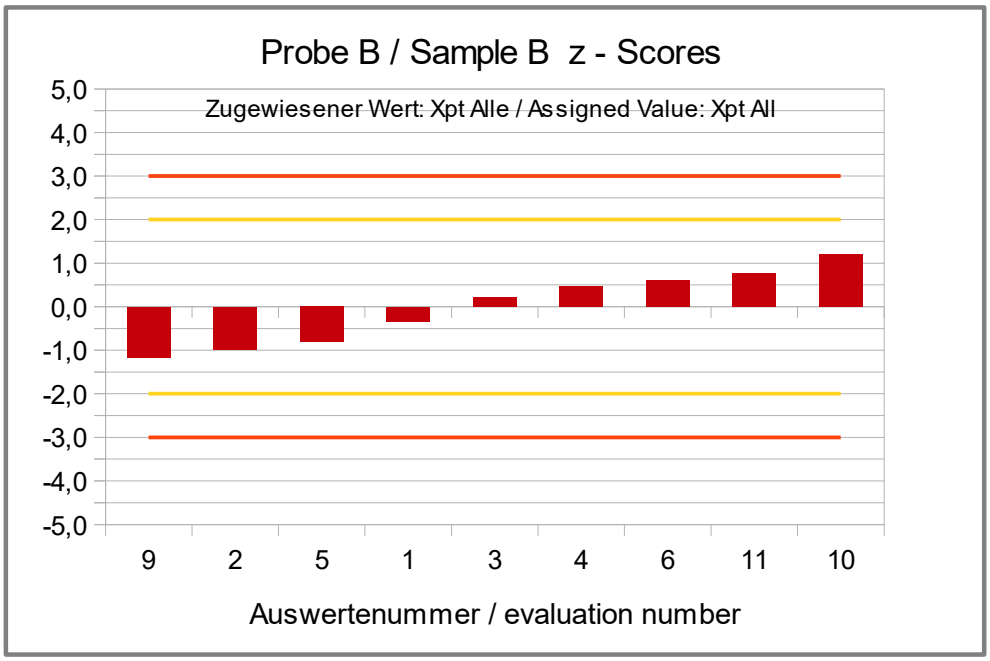

Abb./Fig. 5:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

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Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	33,2	0,26	BF	
10	39,6	1,1	BF	
1	32,1	0,12	BK	
9	22,0	-1,2	BK	
3	26,3	-0,63	MI-II	result converted °
4	34,0	0,36	RS	
6	24,7	-0,83	RS	
7			RS	
8	>6,00		RS	
5	33,8	0,34	RS-F	
11	35,0	0,49	SP	

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

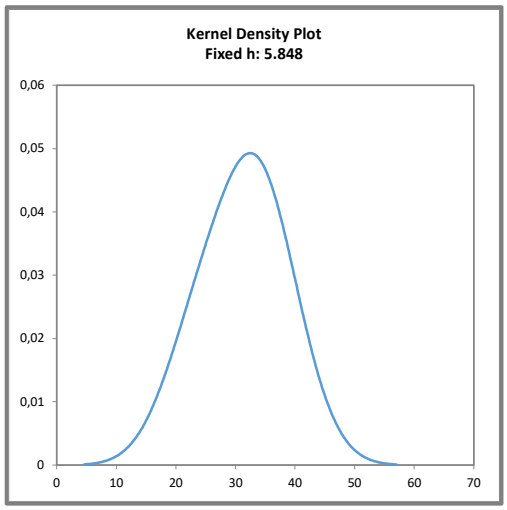

Abb. / Fig. 6:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an almost symmetrical distribution of the results.

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Characteristics: Quantitative evaluation ELISA Peanut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	9
Number of outliers	0
Mean	31,2
Median	33,2
Robust Mean (X_{pt})	**31,2**
Robust standard deviation (S*)	**6,41**
Target range:	
Target standard deviation σ_{pt}	**7,80**
lower limit of target range	**15,6**
upper limit of target range	**46,8**
Quotient S/σ_{pt}*	*0,82*
Standard uncertainty $U_{(Xpt)}$	*2,67*
Results in the target range	*9*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed an approximately symmetrical distribution of the results.

The distribution of the results of all methods showed a low variability. The quotients S/σpt was below 1,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (cf. 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 mean of the evaluation was 197% of the spiking level of peanut to the spiking level sample and were thus above the relevant requirements for the methods used (see 3.4.3 and page 31 "Recovery rates with z-scores ELISA for Peanut").

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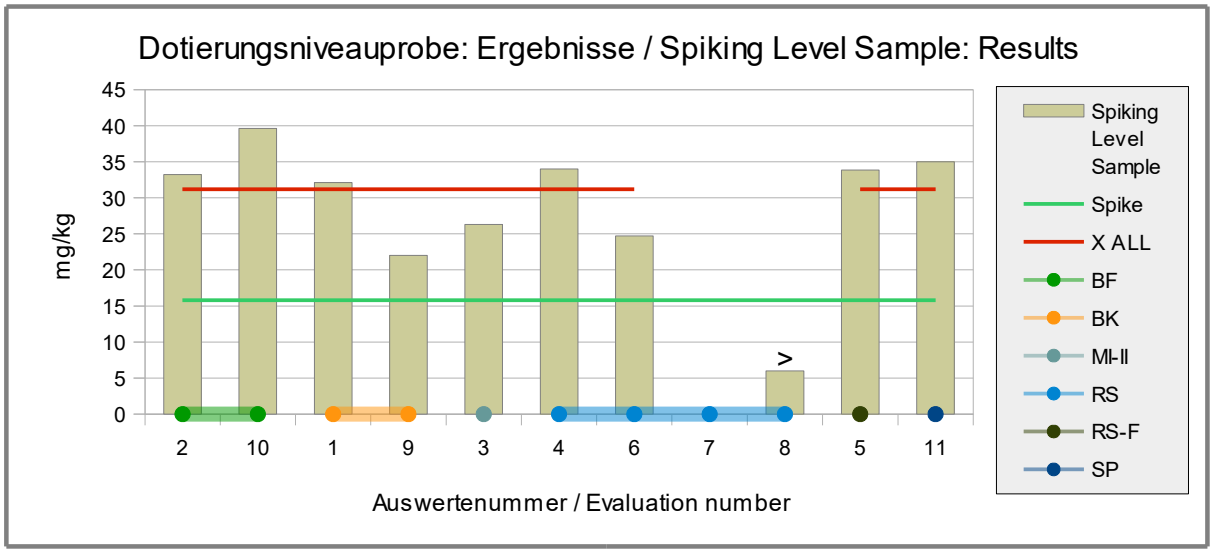

Abb./Fig. 7: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

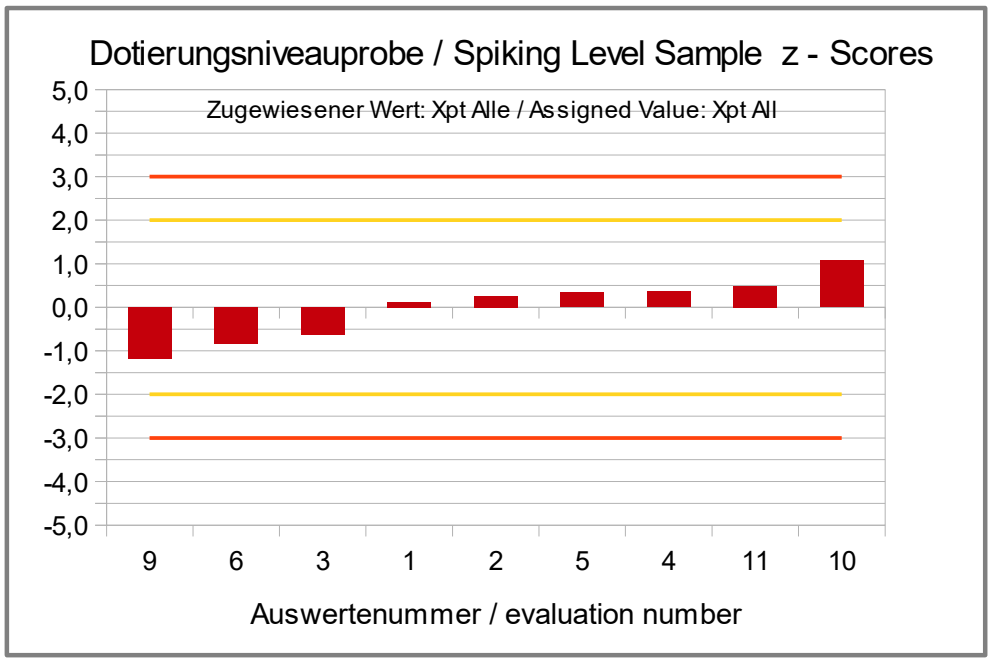

Abb./Fig. 8:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

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Recovery Rates with z-Scores ELISA for Peanut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B–A (Difference)	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
2	33,2	210	4,4	23,4	**91**	-0,36	BF	
10	39,6	251	6,0	40,4	157	2,3	BF	
1	32,1	203	4,1	27,3	**106**	0,25	BK	
9	22,0	**139**	1,6	21,0	**82**	-0,73	BK	
3	26,3	166	2,7	31,1	**121**	0,84	MI-II	result converted °
4	34,0	215	4,6	32,7	**127**	1,1	RS	
6	24,7	156	2,3	34,0	**132**	1,3	RS	
7							RS	
8	>6,00			>6,00			RS	
5	33,8	214	4,6	24,7	**96**	-0,16	RS-F	
11	35,0	222	4,9	35,2	**137**	1,5	SP	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	8
Percent in RA	11	Percent in RA	89

* 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
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comments:

One (11%) participant obtained a recovery rate in the range of the AOAC requirement of 50-150% with the spiking level sample by ELISA. All other recovery rates were above 150%.

For the calculation of the recovery rates of the spiked sample B, the peanut results of the food matrix sample A, where indicated by the participants, were subtracted from the results for sample B. Thus, 89% (8) of the recovery rates for sample B were in the acceptance range of 50-150%.

The related z-scores are based on the target standard deviation of 25%.

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4.1.2 PCR Results: Peanut

Qualitative evaluation 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		
3	positive		positive		2/2 (100%)	ASU	
2	negative	<0,4	positive		1/2 (50%)	SFA-ID	
4	positive		positive		2/2 (100%)	SFA-Q	
1	positive		positive		2/2 (100%)	div	
9	positive		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	4		5	
Number negative	1		0	
Percent positive	80		100	
Percent negative	20		0	
Consensus value	positive		positive	

Methods:
ASU = ASU §64 Methode/method
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 positive consensus value of sample B is in qualitative agreement with the spiking of sample B. A positive consensus value was also obtained for the unspiked sample A.

Qualitative evaluation of PCR Results: Spiking Level Sample

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
3	positive			ASU	
2	positive			SFA-ID	
4	positive			SFA-Q	
1	positive			div	
9	positive			div	

Number positive	5	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

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

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

Quantitative Evaluation PCR: Sample B and Spiking Level Sample

No quantitative results were submitted by the participants.

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4.2 Proficiency Test Almond

4.2.1 ELISA Results: Almond

Qualitative evaluation 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	<1	positive	14,6	2/2 (100%)	BF	
10	negative	<LOD	positive	21,6	2/2 (100%)	BF	
1	negative	< 2,5	positive	17,8	2/2 (100%)	RS-F	
4	negative		positive	16,1	2/2 (100%)	RS-F	
5	negative	<2,5	positive	17,7	2/2 (100%)	RS-F	
6	negative		positive	15,0	2/2 (100%)	RS-F	
7	negative	<2,5	positive	16,0	2/2 (100%)	RS-F	
8	negative	<2,5	positive	16,1	2/2 (100%)	RS-F	
9a	negative	<2,5	positive	25,0	2/2 (100%)	RS-F	
3	negative	<0,4	positive	12,0	2/2 (100%)	SP	
11	negative	<0,4	positive	17,0	2/2 (100%)	SP	
9b	negative	<2,5	positive	15,0	2/2 (100%)	VT	

	Sample A		Sample B	
Number positive	0		12	
Number negative	12		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
BF = MonoTrace ELISA, BioFront Technologies
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

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Quantitative evaluation of ELISA-results: Sample B

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	14,6	-0,48		BF	
10	21,6	1,2		BF	
1	17,8	0,28	0,21	RS-F	
4	16,1	-0,11	-0,17	RS-F	
5	17,7	0,27	0,20	RS-F	
6	15,0	-0,38	-0,44	RS-F	
7	16,0	-0,14	-0,20	RS-F	
8	16,1	-0,11	-0,18	RS-F	
9a	25,0	2,0	1,9	RS-F	
3	12,0	-1,1		SP	
11	17,0	0,10		SP	
9b	15,0	-0,38		VT	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

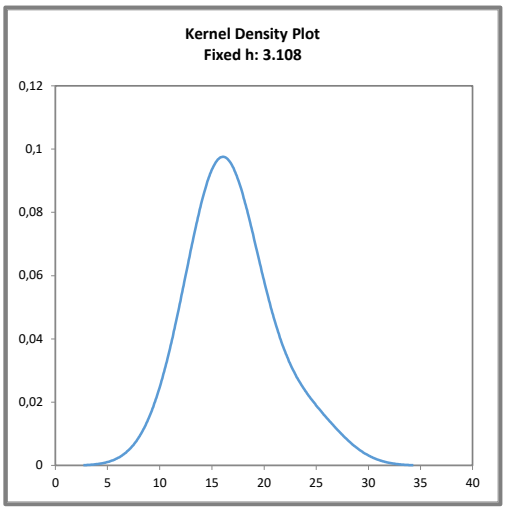

Abb. / Fig. 9:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an almost symmetrical distribution of the results with a slight shoulder at approx. > 22 mg/kg, which can be ascribed to 2 higher single values (methods BF and RS-F).

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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	7
Number of outliers	–	–
Mean	17,0	17,7
Median	16,1	16,1
Robust Mean (X_{pt})	**16,6**	**16,9**
Robust standard deviation (S*)	**2,60**	**1,67**
Target range:		
Target standard deviation σ_{pt}	**4,14**	**4,22**
lower limit of target range	**8,29**	**8,43**
upper limit of target range	**24,9**	**25,3**
Quotient S/σ_{pt}*	*0,63*	*0,40*
Standard uncertainty $U_{(Xpt)}$	*0,939*	*0,791*
Results in the target range	*12*	*7*
Percent in the target range	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution without obvious method-dependent differences.

The evaluation of the results of all methods and method RS-F showed a low variability of the results. The quotient S/σ_{pt} was below 1,0 in each case. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (cf. 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 63% and 64% of the spiking level of almond to sample B and thus within the range of the recommendations for the applied methods (see 3.4.3 and p. 42 "Recovery rates with z-scores ELISA for Almond").

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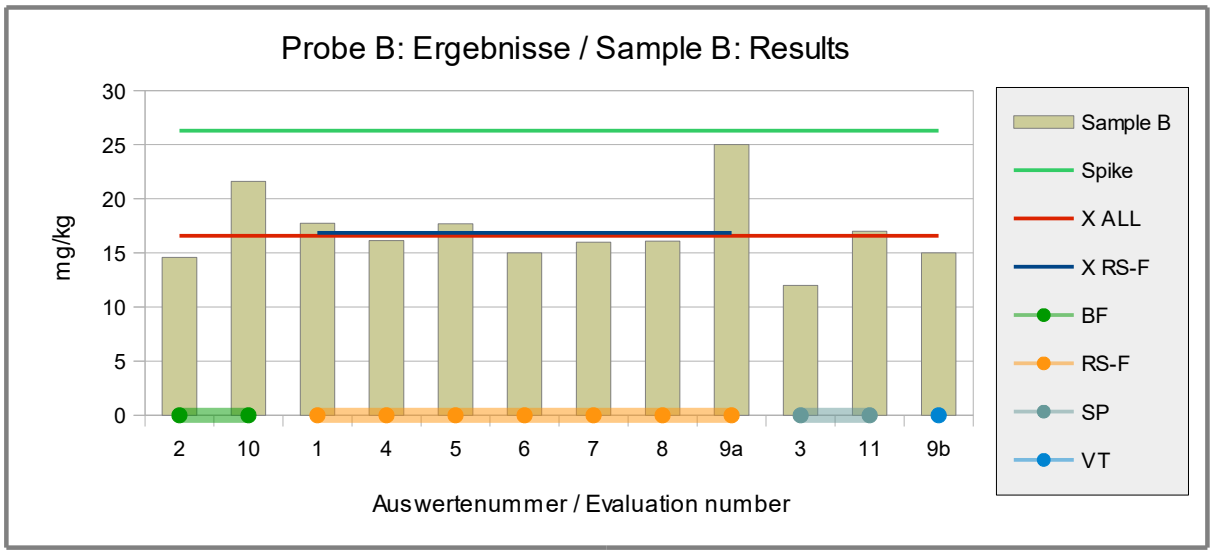

Abb./Fig. 10: ELISA Results Almond
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

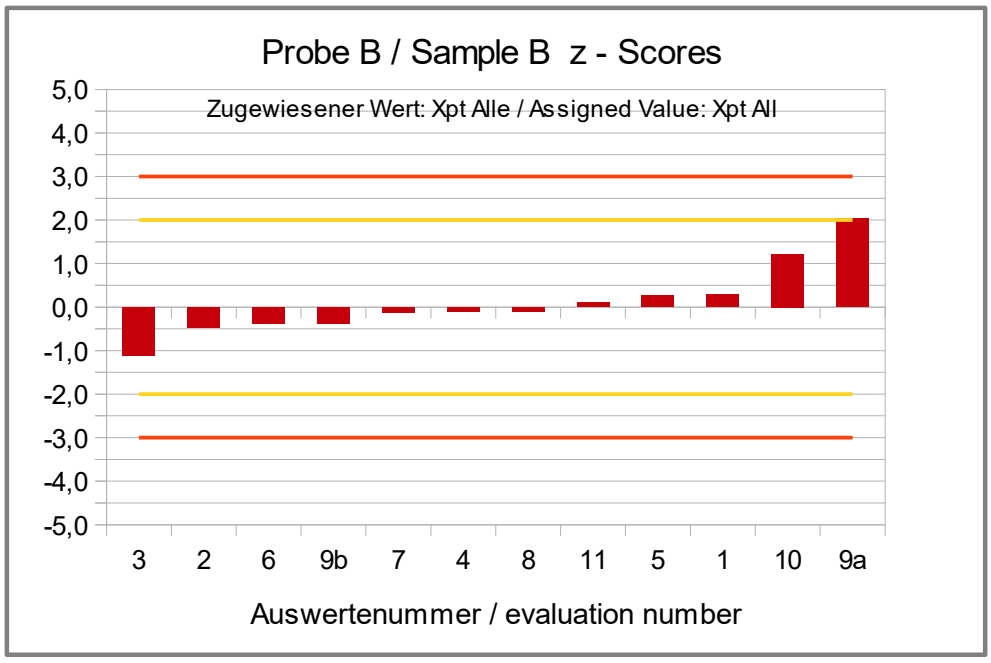

Abb./Fig. 11:
z-Scores for information (ELISA Results Almond)
Assigned value robust mean of all results

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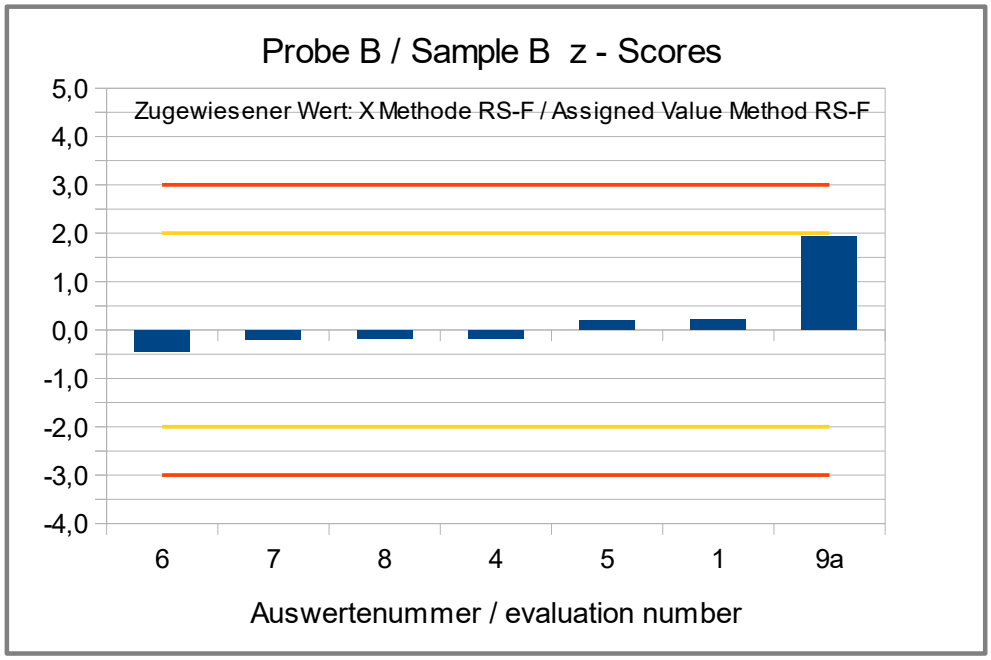

Abb./Fig. 12:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

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Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	15,6	-0,31		BF	
10	23,5	1,6		BF	
1	19,3	0,57	0,38	RS-F	
4	16,0	-0,21	-0,37	RS-F	
5	17,2	0,08	-0,09	RS-F	
6	19,2	0,55	0,36	RS-F	
7	14,0	-0,68	-0,82	RS-F	
8	17,6	0,17	-0,01	RS-F	
9a	20,0	0,74	0,54	RS-F	
3	10,0	-1,6		SP	
11	15,0	-0,45		SP	
9b	15,0	-0,45		VT	

Methods:

BF = MonoTrace ELISA, BioFront Technologies

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

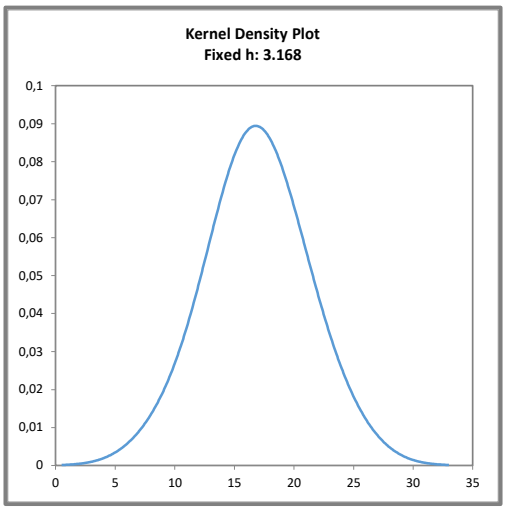

Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows an almost symmetrical distribution of the results.

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Characteristics: Quantitative evaluation ELISA 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	12	7
Number of outliers	0	0
Mean	16,9	17,6
Median	16,6	17,6
Robust Mean (X)	**16,9**	**17,6**
Robust standard deviation (S*)	**3,12**	**2,39**
Target range:		
Target standard deviation σ_{pt}	**4,22**	**4,41**
lower limit of target range	**8,45**	**8,81**
upper limit of target range	**25,3**	**26,4**
Quotient S/σ_{pt}*	*0,74*	*0,54*
Standard uncertainty $U_{(Xpt)}$	*1,13*	*1,13*
Results in the target range	*12*	*7*
Percent in the target range	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed an approximately symmetrical distribution.

The distribution of the results for all methods as well as for method RS-F showed a low variability. The quotients S/σ_{pt} were below 1,0 in each case. 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 88% and 91% of the spiking level of almond to the spiking level sample and were thus within the range of the recommendations for the applied methods (s. 3.4.3 and p. 42 "Recovery rates with z-Scores ELISA for Almond").

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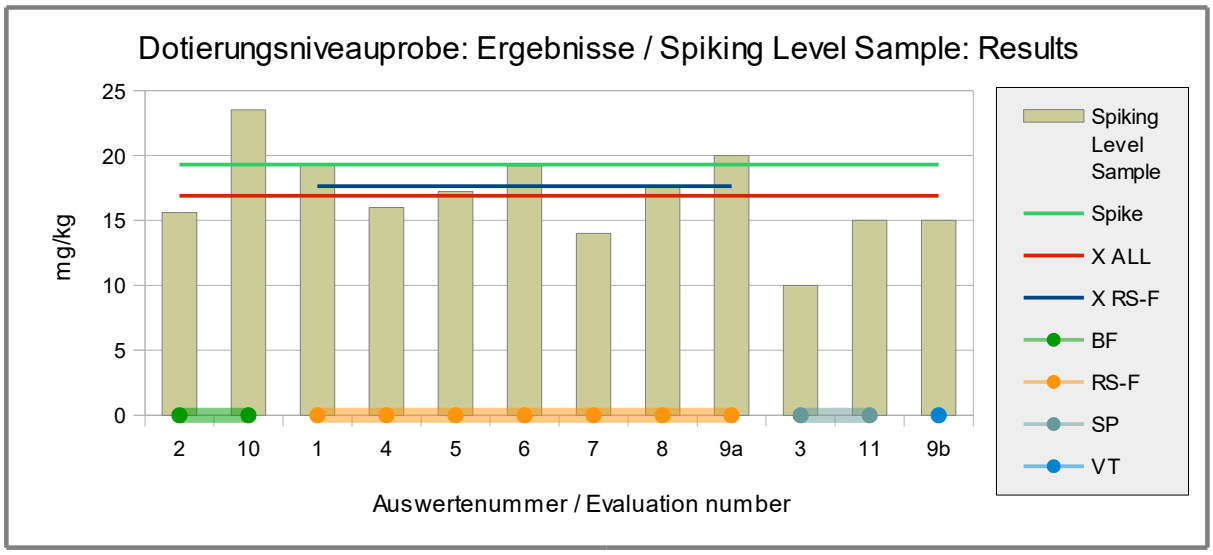

Abb./Fig. 14: ELISA Results Almond
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

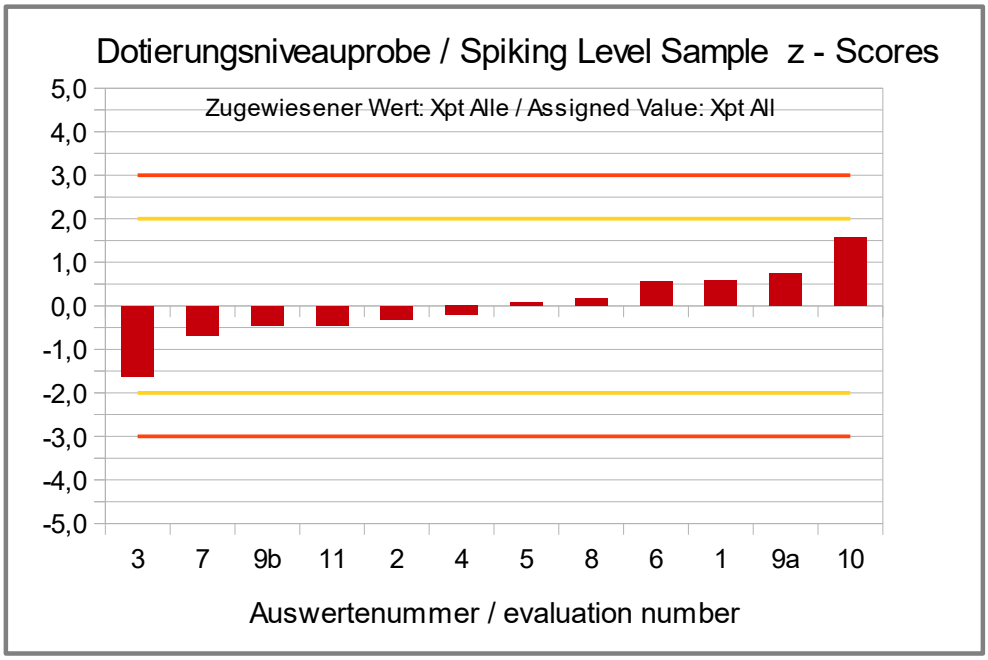

Abb./Fig. 15:
z-Scores (ELISA Results Almond)
Assigned value robust mean of all results

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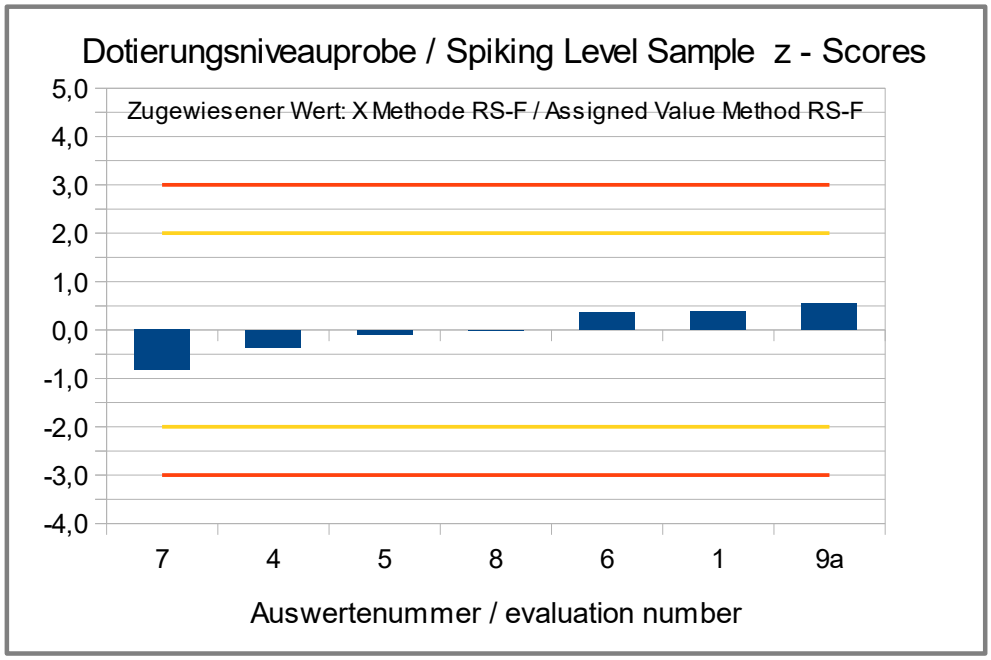

Abb./Fig. 16:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

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Recovery Rates with z-Scores ELISA for Almond: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
2	15,6	**81**	-0,77	14,6	**56**	-1,8	BF	
10	23,5	**122**	0,87	21,6	**82**	-0,71	BF	
1	19,3	**100**	0,00	17,8	**67**	-1,3	RS-F	
4	16,0	**83**	-0,68	16,1	**61**	-1,5	RS-F	
5	17,2	**89**	-0,43	17,7	**67**	-1,3	RS-F	
6	19,2	**99**	-0,02	15,0	**57**	-1,7	RS-F	
7	14,0	**73**	-1,1	16,0	**61**	-1,6	RS-F	
8	17,6	**91**	-0,35	16,1	**61**	-1,6	RS-F	
9a	20,0	**104**	0,15	25,0	**95**	-0,20	RS-F	
3	10,0	**52**	-1,9	12,0	46	-2,2	SP	
11	15,0	**78**	-0,89	17,0	**65**	-1,4	SP	
9b	15,0	**78**	-0,89	15,0	**57**	-1,7	VT	

RA**	50-150 %	RA**	50-150 %
Number in RA	**12**	Number in RA	**11**
Percent in RA	**100**	Percent in RA	**92**

Methods:
BF = MonoTrace ELISA, BioFront Technologies
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
All 12 participants 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 92% (11) of the recovery rates were within this range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

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4.2.2 PCR Results: Almond

Qualitative evaluation 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		
1	negative		positive		1/1 (100%)	SFA	
2	negative	<0,4	positive		1/1 (100%)	SFA-ID	
9	negative		negative		1/1 (100%)	div	

	Sample A		Sample B	
Number positive	0		2	
Number negative	3		1	
Percent positive	0		67	
Percent negative	100		33	
Consensus value	negative		none	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
The negative consensus value for sample A is in qualitative agreement with the spiking of sample B. 2 positive results were reported for sample B, so that no consensus value of ≥ 75% could be determined.

Qualitative evaluation PCR: Spiking Level Sample

Evaluation number	Almond	Almond	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positive			SFA	
2	positive			SFA-ID	
9	positive			div	

Number positive	3	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative evaluation PCR: Sample B and Spiking Level Sample

No quantitative results were submitted by the participants.

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4.3 Participant z-Scores: overview table

Z-Scores for the assigned values from participants' results (consensus values)

Evaluation number	ELISA Peanut: **Xpt** (div. Methods)			ELISA Mandel: **Xpt** (div. Methoden)		ELISA Mandel: **Xpt** (Methode: RS-F)	
	Sample A	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1	-1,6	-0,33	0,12	0,28	0,57	0,21	0,38
2		-0,98	0,26	-0,48	-0,31		
3	-0,36	0,23	-0,63	-1,1	-1,6		
4	0,22	0,46	0,36	-0,11	-0,21	-0,17	-0,37
5		-0,81	0,34	0,27	0,08	0,20	-0,09
6	0,02	0,62	-0,83	-0,38	0,55	-0,44	0,36
7				-0,14	-0,68	-0,20	-0,82
8	0,38			-0,11	0,17	-0,18	-0,01
9 / 9a	-1,8	-1,2	-1,2	2,0	0,74	1,9	0,54
9b				-0,38	-0,45		
10		1,2	1,1	1,2	1,6		
11	0,00	0,77	0,49	0,10	-0,45		

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

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Peanut:		ELISA Almond:	
	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1	0,25	4,1	-1,3	0,00
2	-0,36	4,4	-1,8	-0,77
3	0,84	2,7	-2,2	-1,9
4	1,1	4,6	-1,5	-0,68
5	-0,16	4,6	-1,3	-0,43
6	1,3	2,3	-1,7	-0,02
7			-1,6	-1,1
8			-1,6	-0,35
9 / 9a	-0,73	1,6	-0,20	0,15
9b			-1,7	-0,89
10	2,3	6,0	-0,71	0,87
11	1,5	4,9	-1,4	-0,89

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

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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. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Level 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
BF	2		negative	<1	positive	23,4	positive	33,2		1		Peanut	MonoTrace Peanut ELISA kit, BioFront Technologies
BF	10	24/11	negative	bLOQ	positive	40,36	positive	39,6		1		Please select!	MonoTrace Peanut ELISA kit, BioFront Technologies
BK	1	04+05/10/21	-	1,1	-	28,41	-	32,09		1		Peanut	BioKits Peanut Assay Kit, Neogen
BK	9	27.09.21	positive	1	positive	22	positive	22	1	1		Peanut	BioKits Peanut Assay Kit, Neogen
MI-II	3	20.10.	positive	0,38	positive	7,6	positive	6,1	0,2	0,2		Peanut protein	Peanut ELISA Kit-II, Morinaga
RS	4	28.10.21	positive	1,9	positive	34,6	positive	34	0,75	0,75	30,6	Peanut	others: please select!
RS	6	22.09.21	positive	1,81	positive	35,8	positive	24,7		0,75	50	Peanut	
RS	7	10.11.21	positive		positive		positive			0,75		Please select!	Ridascreen Peanut (R6811), R-Biopharm
RS	8	02.11.21	positive	1,97	positive	>6,00	positive	>6,00	-	0,75		Peanut	Ridascreem peanut R6811
RS-F	5	27.09.21	negative	<2,5	positive	24,69	positive	33,84	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
SP	11	20.09.21	positive	1,8	positive	37	positive	35	0,1	1		Peanut	Eurofins SensiSpec Peanut ELISA Kit

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

Meth. Abbr.	Evaluation number	Specifity	Remarks on the method (extraction and determination)	Method accredited ISO/IEC 17025	Further Remarks
		antibodies	e.g. extraction solution / time / temperature	yes/no	
BF	2			yes	
BF	10	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 60C	no	
BK	1	Ara H1	according to test instructions	yes	
BK	9				Sample A detected at the LOD
MI-II	3	detects peanut proteins	according to manufacturer information	yes	Peanut Sensitive ELISA Kit II, M2120
RS	4			yes	Ridacreen Peanut R6811
RS	6			yes	Kit: RIDASCREEN® Peanut ELISA Kit (Art. Nr.: R6811)
RS	7			yes	
RS	8		diluted allergen extaction buffer / 10min / 60°C	no	
RS-F	5	Peanut proteins	according to test instructions	yes	Verification with Ridascreen Peanut R6811
SP	11				

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5.1.2 ELISA: Almond

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Level 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
BF	2		negative	<1	positive	14,6	positive	15,6		1		Almond	MonoTrace Almond ELISA kit, BioFront Technologies
BF	10	24/11	negative	bLOD	positive	21,6	positive	23,5	0,15	1		Please select!	MonoTrace Almond ELISA kit, BioFront Technologies
RS-F	1	13.10.21	-	< 2,5	-	17,75	-	19,31		2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	4	28.10.21	negative		positive	16,13	positive	16	2,5	2,5	31,3	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	5	27.09.21	negative	<2,5	positive	17,69	positive	17,23	0,1	2,5		Almond	Ridascreen Fast Mandel (R6901), r-Biopharm
RS-F	6	22.09.21	negative		positive	15	positive	19,2		2,5	40	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	7	04.11.21	negative	<2,5	positive	16	positive	14		2,5		Please select!	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	8	04.11.21	negative	<2,5	positive	16,1	positive	17,6	-	2,5			Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	9a	30.09.21	negative	<2.5	positive	25	positive	20	2,5	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
SP	3	23.9.	negative	<0,4	positive	12	positive	10	0,4	0,4		Almond	Eurofins SensiSpec Almond ELISA Kit
SP	11	20.09.21	negative	< 0.4	positive	17	positive	15	0,1	0,4		Almond	Eurofins SensiSpec Almond ELISA Kit
VT	9b	29.09.21	negative	<2.5	positive	15	positive	15	2,5	2,5		Almond	Veratox Almond, Neogen

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

Meth. Abbr.	Evaluation number	Specifity	Remarks on the method (extraction and determination)	Method accredited ISO/IEC 17025	Further Remarks
		antibodies	e.g. extraction solution / time / temperature	yes/no	
BF	2			yes	
BF	10	monoclonal antibody-based kit	1:20 extraction ratio, 10 minutes at 60C	no	
RS-F	1	Ab against almond protein	according to test instructions	yes	
RS-F	4			yes	
RS-F	5	Almond proteins	according to test instructions	yes	
RS-F	6			yes	
RS-F	7			no	
RS-F	8		diluted allergen extaction buffer / 10min / 60°C	yes	
RS-F	9a				
SP	3	detects almond proteins	according to manufacturer information	yes	HU0030025/HU0030001
SP	11				
VT	9b				

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5.1.3 PCR: Peanut

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Level 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 / food protein	Test-Kit + Manufacturer
ASU	3	13.10.	positive		positive		positive		10			Peanut DNA	ASU §64 Methode/method
SFA-ID	2		negative	<0,4	positive		positive		0,4			Peanut DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	4	21.09.21	positive		positive		positive		0,4			Peanut	Sure Food Allergen Quant, R-Biopharm / Congen
div	1	27.09.21	positive		positive		positive					Peanut DNA	Selection PCR-Methods
div	9	27.09.21	positive		positive		positive					Peanut DNA	Selection PCR-Methods

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

Meth. Abbr.	Evaluation number	Specifity	Remarks on 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	3		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Realtime PCR / 45 cycles	yes	§ 64 LFGB L 00.00-169:2019-07
SFA-ID	2			yes	
SFA-Q	4			yes	
div	1		Spiking Level Sample: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles Samples A+B: Dneasy Mericon Food-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
div	9				Sample A detected at the LOD

5.1.4 PCR: Almond

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Level 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
SFA	1	22.09.21	negative		positive		positive					Almond-DNA	SureFood ALLERGEN, r-biopharm/Congen
SFA-ID	2		negative	<0,4	positive		positive		0,4			Almond-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	9	15.10.21	negative		negative		positive					Almond-DNA	

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

Meth. Abbr.	Evaluation number	Specifity	Remarks on 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	
SFA	1	characteristic sequence section of almond DNA	Spiking Level Sample: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles Samples A+B: Dneasy Mericon Food-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
SFA-ID	2			yes	
div	9				Sample B signals <LOD (10 copies)

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5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL09 Sample B

Weight whole sample	2,51	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,5	mg/kg

Result of analysis

Sample	Weight (g)	Particle number	Particles [mg/kg]
1	4,98	83	33,3
2	4,98	83	33,3
3	5,01	81	32,3
4	5,01	70	27,9
5	5,00	80	32,0
6	5,00	83	33,2
7	5,00	73	29,2
8	4,97	62	24,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	76,9	Particle
Standard deviation	7,74	Particle
χ^2 (CHI-Quadrat)	5,46	
Probability	**60**	%
Recovery rate	108	%

Normal distribution		
Number of samples	8	
Mean	30,8	mg/kg
Standard deviation	3,10	mg/kg
rel. Standard deviaton	10,1	%
Horwitz standard deviation	9,6	%
HorRat-value	**1,1**	
Recovery rate	108	%

Microtracer Homogeneity Test
DLA ptAL09 Spiking Level Sample

Weight whole sample	1,50	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	55	22,0
2	4,98	56	22,5
3	5,02	61	24,3
4	4,99	63	25,3
5	5,02	54	21,5
6	4,97	54	21,7
7	5,03	61	24,3
8	5,02	51	20,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	56,9	Particle
Standard deviation	4,24	Particle
χ^2 (CHI-Quadrat)	2,21	
Probability	**95**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	22,7	mg/kg
Standard deviation	1,69	mg/kg
rel. Standard deviaton	7,5	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,75**	
Recovery rate	105	%

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5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL09 - 2021***
PT name	***Allergens IX: Peanut and Almond in Barbecue Spice Mixture (with Onion, Garlic and Paprika powder) with "Spiking Level Sample“***
Sample matrix (processing)	***Samples A + B:*** *Barbecue Spice Mixture / ingredients: paprika powder, onion powder, garlic powder, 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 + Spiking Level Sample:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Peanut (Peanutprotein, DNA), Almond (Almondprotein, 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***
Last Deadline	***the latest November 12th 2021***
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.*

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6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		SPAIN
		Germany
		USA
		SWITZERLAND
		ITALY
		Germany
		ITALY
		Germany
		Germany
		Germany

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

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

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