Evaluation Report
proficiency test

DLA ptAL05 (2020)

Allergens V:

Peanut and Almond

in Pastry (Cocoa Biscuit)

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

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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.

Contents

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 common in commerce cocoa bicuits. 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 containing the allergenic ingredients peanut and almond, and then dried (50°C, overnight). After crushing, sieving (mesh 1,0 mm) and homogenization the baked cookies containing the allergenic ingredients were added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in two additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

The samples A and B were portioned to approximately 25 g, the spiking level sample to approximately 15 g in metallized PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Cocoa Biscuits Ingredients: Wheat flour, sugar, palm oil, glucose syrup, low-fat cocoa powder (3.8%), raising agents: sodium carbonates, diphosphates; Salt, apple extract, skimmed milk powder, emulsifier: lecithins (soy); Flavors, acidulants: citric acid; Starch (wheat), whole egg powder. Nutrients per 100 g: Fat 14 g, Carbohydrates 69 g, Protein 7,6 g	100 g/100 g	94,5 g/100g	-
Cookies (baked 150°C, 30 min) Ingredients: Wheat flour, sugar, butter, eggs, cocoa powder and peanuts, almonds and further ingredients (see below)	-	5,47 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,9 g/100 g
Peanuts, roasted milled, mixture (18 products from USA, Asia, Africa, South America) - as Peanut* - *thereof 23,2% total protein***	-	 30,4 mg/kg 7,06 mg/kg	 21,3 mg/kg 4,94 mg/kg
Almond, roasted milled, mixture (23 products from USA, Europe, Australia, Middle East) - as almond* - *thereof 21,1% total protein***	-	 33,5 mg/kg 7,07 mg/kg	 20,2 mg/kg 4,27 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=5,46 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 samples B and the spiking level sample showed a probability of 92% and 98%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value 0,87 or 0,65. 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 and AgraQuant) and almond (Immunolab 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].

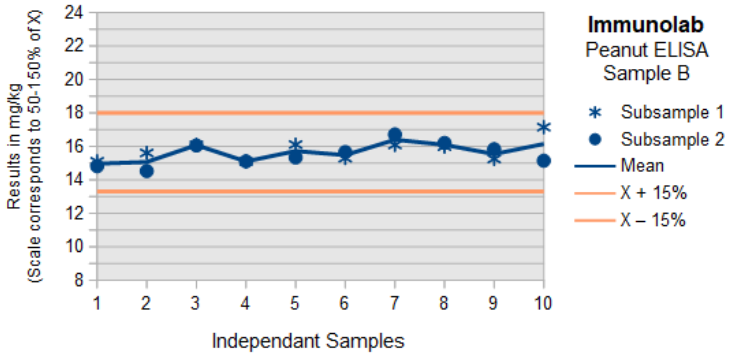
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 15,7 ± 0,42 mg/kg

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

General average X	15,7	
SD of sample means Sx	0,51	3,3%
SD within-samples Sw	0,41	2,6%
SD between-samples Ss	0,42	2,7%

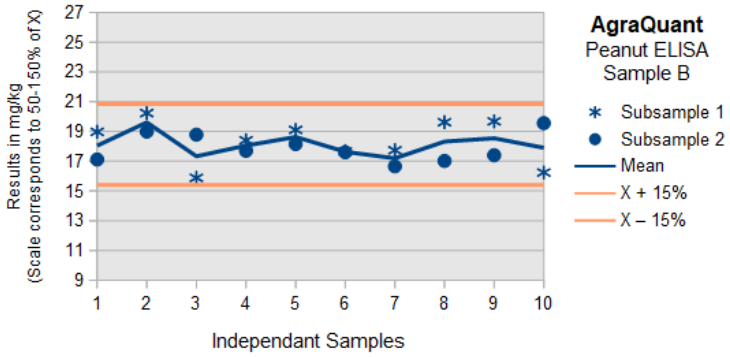


AgraQuant Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 18,1 ± 0,10 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	19,0	17,1	18,0
2	20,2	19,0	19,6
3	15,9	18,8	17,3
4	18,4	17,7	18,0
5	19,1	18,2	18,6
6	17,7	17,6	17,6
7	17,7	16,7	17,2
8	19,6	17,0	18,3
9	19,7	17,4	18,5
10	16,2	19,6	17,9

General average X	18,1	
SD of sample means Sx	0,70	3,9%
SD within-samples Sw	0,99	5,4%
SD between-samples Ss	0,10	0,5%



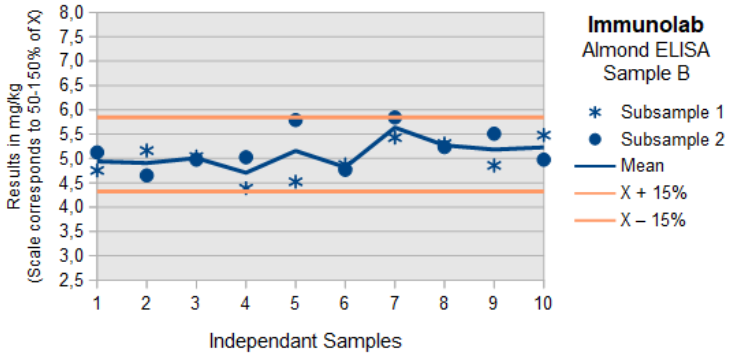
ELISA-Tests: Homogenität Mandel / Homogeneity Almond

Immunolab Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 5,09 ± 0,17 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	4,75	5,12	4,94
2	5,16	4,65	4,91
3	5,04	4,98	5,01
4	4,39	5,02	4,71
5	4,52	5,79	5,16
6	4,88	4,77	4,82
7	5,43	5,84	5,64
8	5,31	5,23	5,27
9	4,86	5,51	5,18
10	5,48	4,98	5,23

General average X	5,09	
SD of sample means Sx	0,27	5,3%
SD within-samples Sw	0,29	5,6%
SD between-samples Ss	0,17	3,4%

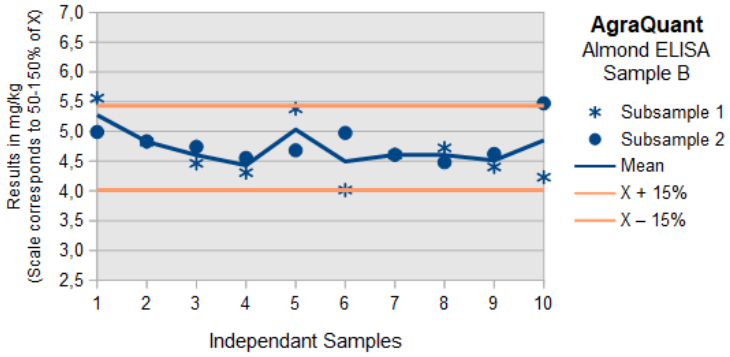


AgraQuant Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 4,72 ± 0,17 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	5,56	4,99	5,28
2	4,82	4,83	4,83
3	4,46	4,74	4,60
4	4,31	4,55	4,43
5	5,38	4,68	5,03
6	4,02	4,98	4,50
7	4,61	4,61	4,61
8	4,72	4,48	4,60
9	4,40	4,62	4,51
10	4,23	5,47	4,85

General average X	4,72	
SD of sample means Sx	0,27	5,7%
SD within-samples Sw	0,30	6,3%
SD between-samples Ss	0,17	3,6%



2.1.2 Stability

The pap samples are preparations preserved with sorbic acid. The stability of the sample material was thus guaranteed during the investigation period under the specified storage conditions.
A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,33 and 0,43 (25°C), respectively. 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 2020. The testing method was optional. The tests should be finished at 6th November 2020 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 **cocoa biscuit**. 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 15 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 methods for the quantitative determination of allergens:

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

i) ***Robust standard deviation of all results*** - S^*_{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]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 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%.

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]**	**Recovery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Peanut	Rice cookie	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 cookie (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 cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR multiplex ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR multiplex ASU 18.00-22
Brazil Nut	Rice cookie	89,1 17,3 9,8	89,1 % 86,5 % 98 %	-	34,1% 36,2% 40,2%	34,4% 38,2% 41,8%	24,5% 28,4% 30,6%	rt-PCR ASU 18.00-21
Brazil Nut	Wheat cookie Sauce powder	80,8 42,6	65,7 % 42,6 %	-	25,6% 27,5%	36,4% 39,7%	31,6% 34,6%	rt-PCR ASU 18.00-21
Brazil Nut	Rice cookie	96,6 14,2	96,6 % 71 %	-	16,8% 54,2%	31,8% 56,5%	29,5% 41,5%	rt-PCR multiplex ASU 18.00-22
Brazil Nut	Wheat cookie Sauce powder	76,5 48,4	62,2 % 48,4 %	-	15,6% 34,4%	35,8% 37,5%	34,1% 28,5%	rt-PCR multiplex ASU 18.00-22

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

Literature [18-24]	**Recovery rate**	**Repeatability standard deviation**	**Reproducibility standard deviation**
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% (a)	19,5 - 57,2% (a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

(a) = Example from an hypothetical proficiency scheme in the range of 0,5 - 5 mg/kg

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

3.6 z'-Score

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

The calculation is performed by:

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

If carried out an evaluation of the results by means of z'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.
The requirements for the analytical performance are generally considered as fulfilled if

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

If $U_{(xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.
The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of llergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.
The corresponding z-scores were calculated according to 3.5 with the target standard deviation of 25% (see 3.4.3).

4. Results

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

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

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

ELISA results given as **peanut protein** or **almond protein** were converted by DLA to **total food items (peanut, almond)** using the analyzed protein content of the raw materials (see page 5).

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

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

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

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

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

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

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

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

4.1 Proficiency Test Peanut

4.1.1 ELISA Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
11	negative	<1	positive	13,2	2/2 (100%)	BC	
2a	negative	<1	positive	10,2	2/2 (100%)	BK	
8	negative	<0,86	positive	13,4	2/2 (100%)	MI-II	Result converted °
3	negative		positive	15,6	2/2 (100%)	RS-F	
4	negative		positive	21,0	2/2 (100%)	RS-F	
5	negative	<2,5	positive	20,0	2/2 (100%)	RS-F	
6	negative		positive	20,0	2/2 (100%)	RS-F	
10	negative	<2,5	positive	14,3	2/2 (100%)	RS-F	
13	negative	<2,5	positive	21,3	2/2 (100%)	RS-F	
14	negative		positive	19,2	2/2 (100%)	RS-F	
15	negative	<1	positive	17,0	2/2 (100%)	RS-F	
1	negative	0	positive	22,0	2/2 (100%)	SP	
2b	negative	<2,5	positive	15,6	2/2 (100%)	VT	
12	negative		positive	4,39	2/2 (100%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	0		14	
Number negative	14		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

BC = BioCheck ELISA
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
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
	[mg/kg]				
11	13,2	-0,82		BC	
2a	10,2	-1,5		BK	
8	13,4	-0,78		MI-II	Result converted °
3	15,6	-0,24	-0,64	RS-F	
4	21,0	1,1	0,53	RS-F	
5	20,0	0,82	0,31	RS-F	
6	20,0	0,82	0,31	RS-F	
10	14,3	-0,56	-0,92	RS-F	
13	21,3	1,1	0,58	RS-F	
14	19,2	0,63	0,14	RS-F	
15	17,0	0,11	-0,33	RS-F	
1	22,0	1,3		SP	
2b	15,6	-0,24		VT	
12	4,39	-2,9		VT	

° calculation see p. 19

Methoden:

BC = BioCheck ELISA
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

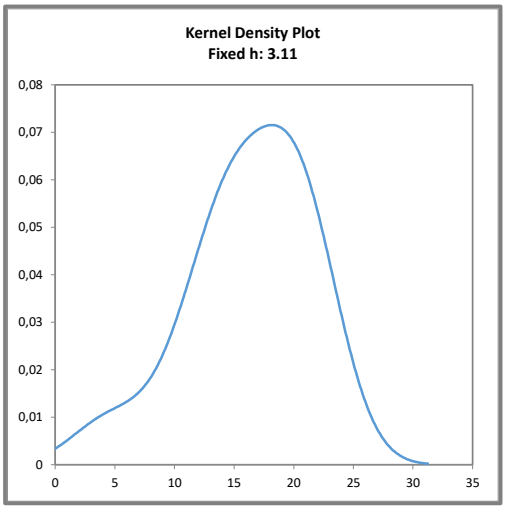


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a slight shoulders below 7 mg/kg, due to an value outside the target range.

Characteristics: Quantitative evaluation ELISA Peanut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	14	8
Number of outliers	0	0
Mean	16,2	18,5
Median	16,3	19,6
Robust Mean (X_{pt})	**16,6**	**18,5**
Robust standard deviation (S*)	**4,66**	**2,94**
Target range:		
Target standard deviation σ_{pt}	**4,15**	**4,64**
lower limit of target range	**8,30**	**9,27**
upper limit of target range	**24,9**	**27,8**
Quotient S/σ_{pt}*	*1,1*	*0,63*
Standard uncertainty $U_{(Xpt)}$	*1,56*	*1,30*
Results in the target range	*13*	*8*
Percent in the target range	*93*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density showed almost a symmetrical distribution.

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

The robust means of the evaluations were 55% and 61% of the spiking level of peanut to sample B and thus in the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for Peanut").

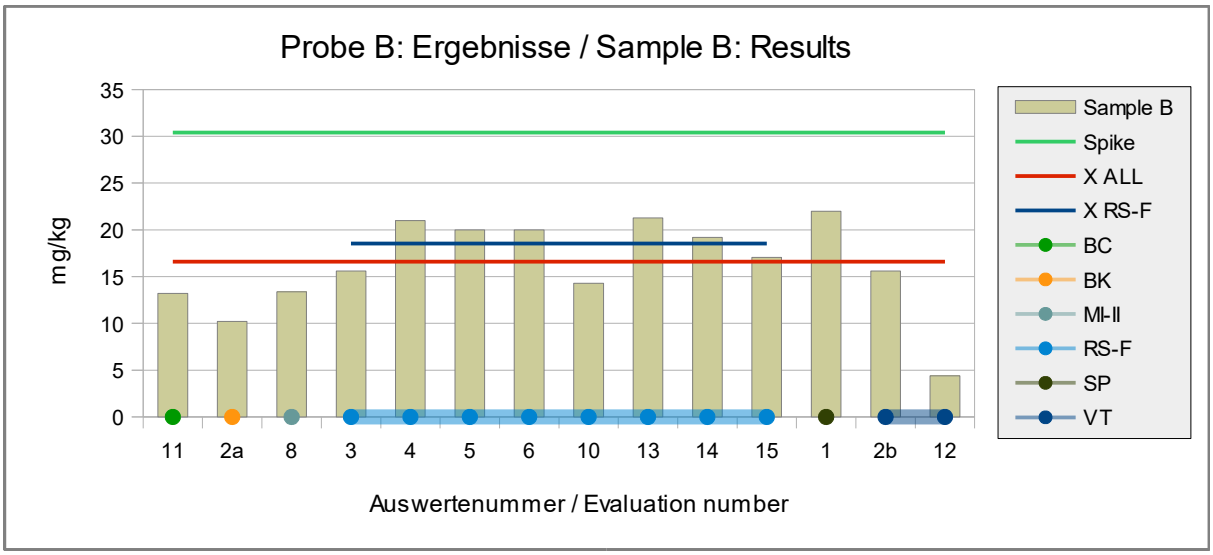


Abb./Fig. 2: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

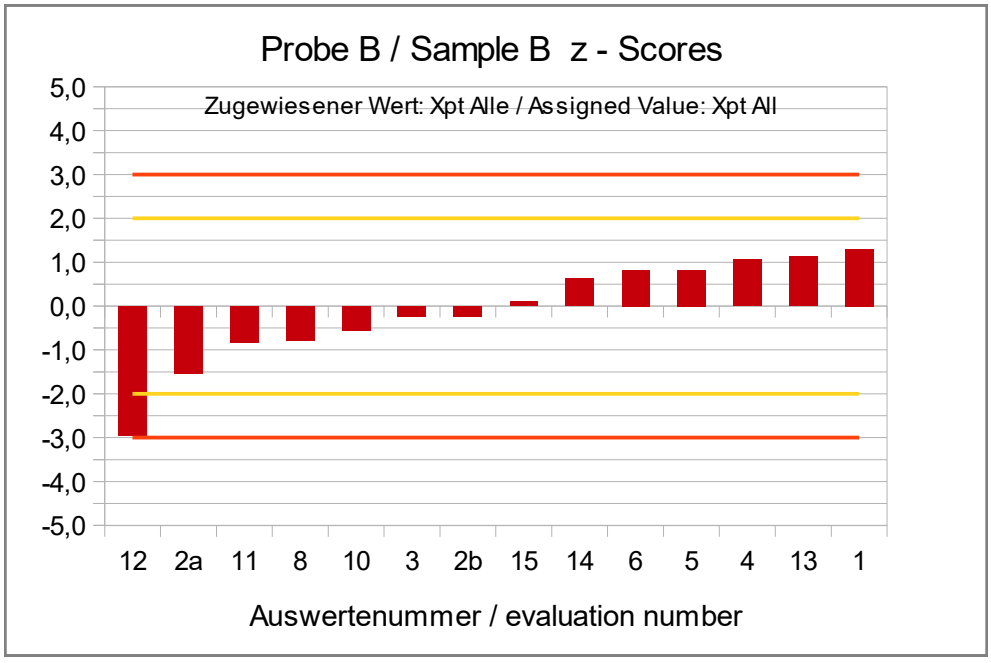


Abb./Fig. 3:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

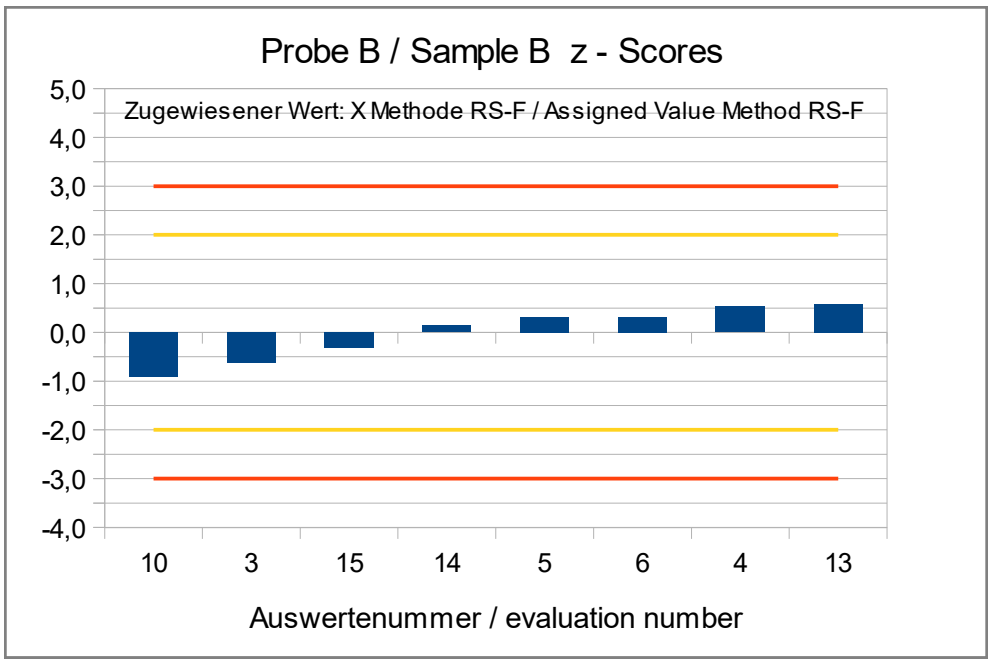


Abb./Fig. 4:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
11	39,9	-0,83		BC	
2a	38,0	-0,98		BK	
8	34,1	-1,3		MI-II	Result converted °
3	49,6	-0,06	-0,41	RS-F	
4	52,0	0,14	-0,23	RS-F	
5	44,7	-0,45	-0,76	RS-F	
6	58,0	0,61	0,20	RS-F	
10	45,4	-0,39	-0,71	RS-F	
13	69,6	1,5	1,0	RS-F	
14	58,8	0,68	0,26	RS-F	
15	63,9	1,1	0,62	RS-F	
1	56,0	0,45		SP	
2b	64,8	1,2		VT	
12	8,87	-3,3		VT	

° calculation see p. 19

Methoden:

BC = BioCheck ELISA
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

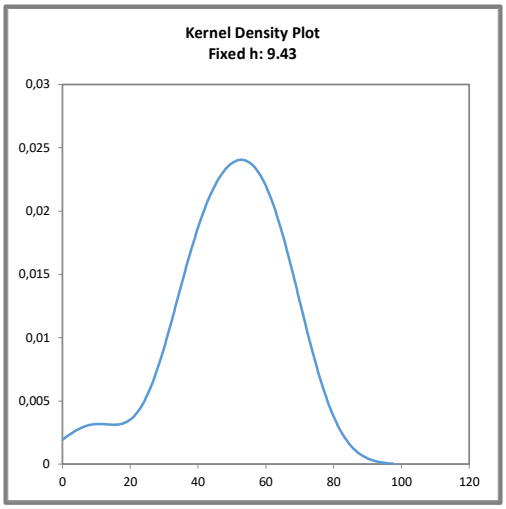


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a small side peak at 9 mg/kg, due to a single value outside the target range.

Characteristics: Quantitative evaluation ELISA Peanut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	14	8
Number of outliers	0	0
Mean	48,8	55,2
Median	50,8	55,0
Robust Mean (X_{pt})	**50,3**	**55,2**
Robust standard deviation (S*)	**13,9**	**10,1**
Target range:		
Target standard deviation σ_{pt}	**12,6**	**13,8**
lower limit of target range	**25,1**	**27,6**
upper limit of target range	**75,4**	**82,9**
Quotient S/σ_{pt}*	*1,1*	*0,73*
Standard uncertainty $U_{(Xpt)}$	*4,63*	*4,44*
Results in the target range	*13*	*8*
Percent in the target range	*93*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution.

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

The robust means of the evaluations were 236% and 259% of the spiking level of peanut to the spiking level sample and were above the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for Peanut").

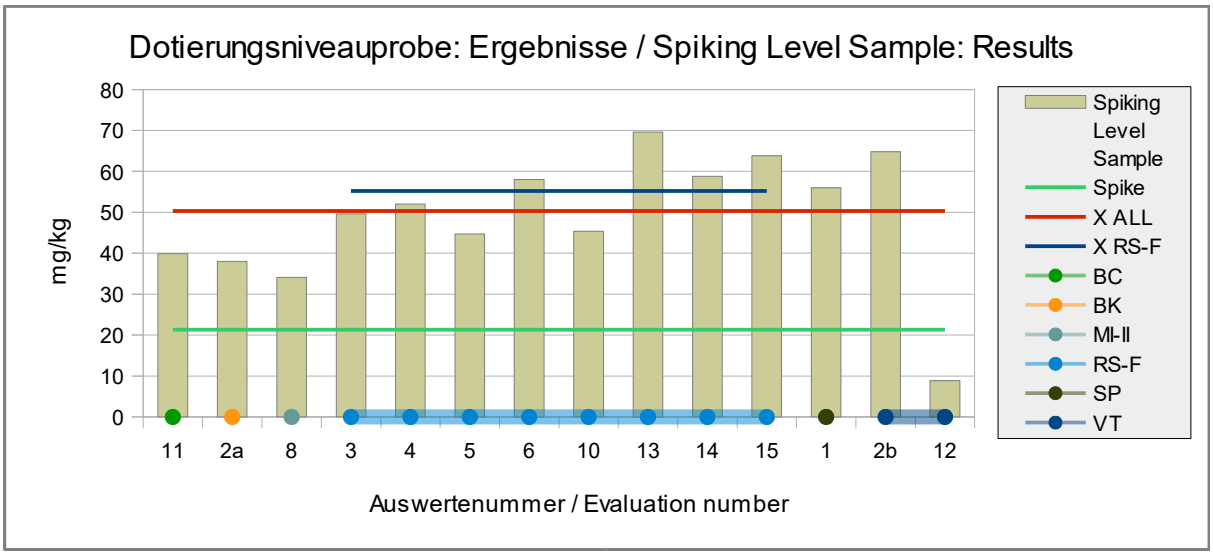


Abb./Fig. 6: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

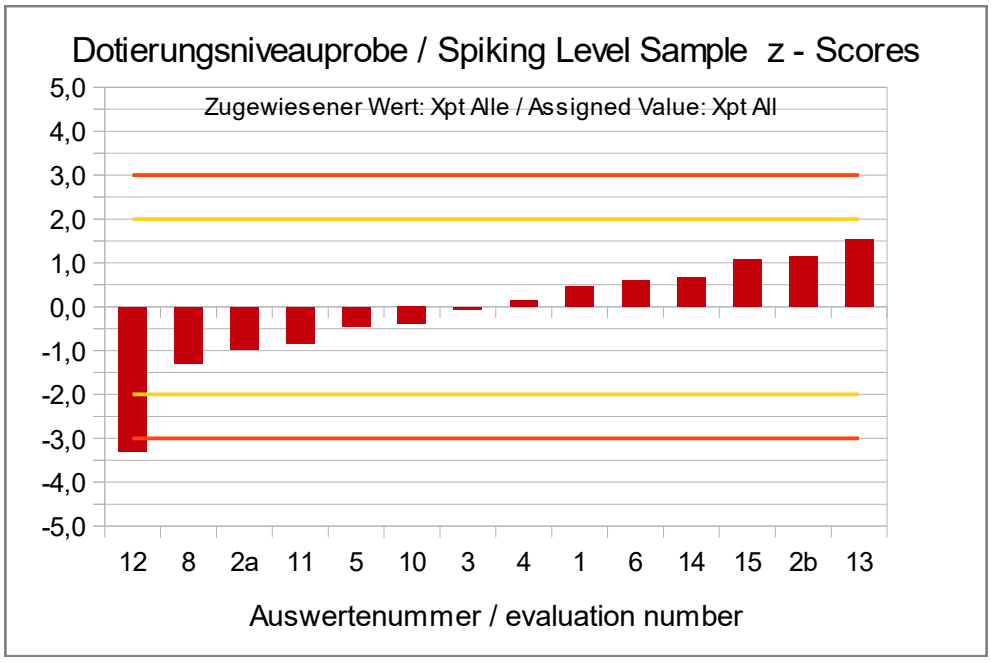


Abb./Fig. 7:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

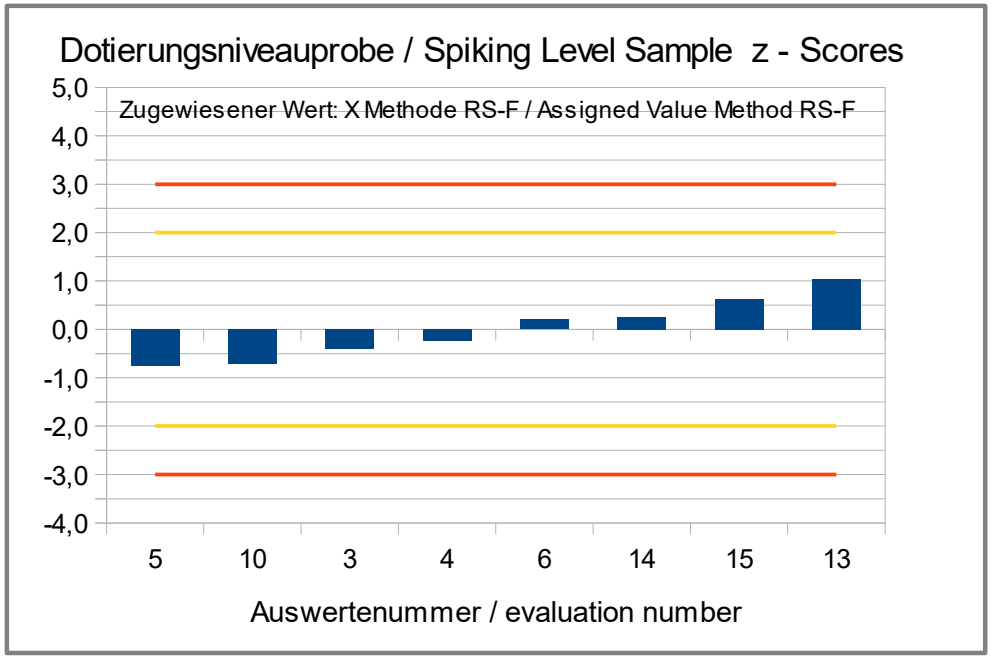


Abb./Fig. 8:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates with z-Scores ELISA for Peanut: 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}]		
11	39,9	187	3,5	13,2	43	-2,3	BC	
2a	38,0	178	3,1	10,2	34	-2,7	BK	
8	34,1	160	2,4	13,4	44	-2,2	MI-II	Result converted °
3	49,6	233	5,3	15,6	**51**	-1,9	RS-F	
4	52,0	244	5,8	21,0	**69**	-1,2	RS-F	
5	44,7	210	4,4	20,0	**66**	-1,4	RS-F	
6	58,0	272	6,9	20,0	**66**	-1,4	RS-F	
10	45,4	213	4,5	14,3	47	-2,1	RS-F	
13	69,6	327	9,1	21,3	**70**	-1,2	RS-F	
14	58,8	276	7,0	19,2	**63**	-1,5	RS-F	
15	63,9	300	8,0	17,0	**56**	-1,8	RS-F	
1	56,0	263	6,5	22,0	**72**	-1,1	SP	
2b	64,8	304	8,2	15,6	**51**	-1,9	VT	
12	8,87	42	-2,3	4,39	14	-3,4	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**9**
Percent in RA	**0**	Percent in RA	**64**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BC = BioCheck ELISA
BK = BioKits, Neogen
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

For the spiking level sample, all recovery rates obtained by ELISA methods were well above the AOAC requirement of 50-150% (exception result no. 12). For the processed spiked food matrix sample B, all recovery rates were below 100%, of which 64% (9) were within the acceptance range.

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

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		
7	negative		negative		1/2 (50%)	SFA	no positive sample detected
15	negative		positive		2/2 (100%)	SFA	
3	negative		positive		2/2 (100%)	div	
8	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		3	
Number negative	4		1	
Percent positive	0		75	
Percent negative	100		25	
Consensus value	negative		positive	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

No quantitative results were submitted.

Quantitative Valuation PCR: Spiking Level Sample

No quantitative results were submitted.

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positive			SFA	
15	positive			SFA	
3	positive			div	
8	positive			div	

Number positive	4
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

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		
3	negative		positive	12,8	2/2 (100%)	AQ	Result converted °
11	negative	<0,5	positive	3,30	2/2 (100%)	BC	
4	negative		positive	12,0	2/2 (100%)	RS-F	
5	negative	<2,5	positive	12,1	2/2 (100%)	RS-F	
6	negative		positive	12,0	2/2 (100%)	RS-F	
10	negative	<2,5	positive	8,16	2/2 (100%)	RS-F	
13	negative	<2,5	positive	9,15	2/2 (100%)	RS-F	
15	negative	<2,5	positive	9,72	2/2 (100%)	RS-F	
1	negative	0	positive	5,00	2/2 (100%)	SP	
8	negative	<0,4	positive	3,60	2/2 (100%)	SP	
2	negative	<2,5	positive	4,40	2/2 (100%)	VT	
9	positive	0,200	positive	6,20	1/2 (50%)	VT	Sample A < LOQ

° calculation see p. 19

	Sample A		Sample B	
Number positive	1		12	
Number negative	11		0	
Percent positive	8		100	
Percent negative	92		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

The consensus values are in qualitative agreement with the spiking of sample B.
For sample A, a positive result below the limit of quantification of method VT (Veratox, Neogen) was given.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Sample B	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]	Info #			
3	12,8	1,8		AQ	Result converted °
11	3,30	-1,9		BC	
4	12,0	1,5	0,56	RS-F	
5	12,1	1,5	0,60	RS-F	
6	12,0	1,5	0,56	RS-F	
10	8,16	-0,02	-0,90	RS-F	
13	9,15	0,38	-0,52	RS-F	
15	9,72	0,60	-0,30	RS-F	
1	5,00	-1,3		SP	
8	3,60	-1,8		SP	
2	4,40	-1,5		VT	
9	6,20	-0,79		VT	

for information only

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

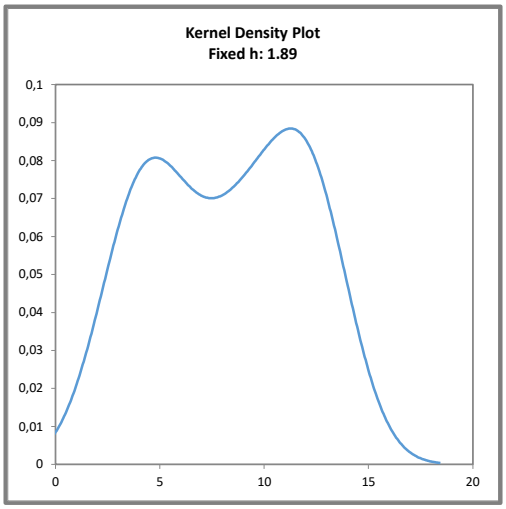


Abb. / Fig. 9:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimate shows a distribution of the results with a maximum at 4,8 mg/kg, which is based on 1-2 results each of methods BC, SP and VT, and a maximum at 11,5 mg/kg, which is based on results of methods AQ and RS-F.

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	0	0
Mean	8,20	10,5
Median	8,66	10,9
Robust Mean (X_{pt})	**8,20**	**10,5**
Robust standard deviation (S*)	**4,08**	**1,96**
Target range:	*Info #*	
Target standard deviation $\sigma_{pt'}$ or σ_{pt}	**2,52**	**2,63**
lower limit of target range	**3,15**	**5,26**
upper limit of target range	**13,2**	**15,8**
Quotient S/$\sigma_{pt'}$ or S*/$\sigma_{pt'}$*	*1,6*	*0,75*
Standard uncertainty $U_{(Xpt)}$	*1,47*	*1,00*
Results in the target range	*12*	*6*
Percent in the target range	*100*	*100*

 # for information only

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a bimodal distribution with possibly method-dependent differences. There were ≥ 5 individual results available for method RS-F, so that a separate evaluation was possible. For the other methods only 1-2 results were available. Thus no separate evaluations could be carried out. Therefore, despite the two-peak distribution, a purely informative evaluation of all methods was carried out. The resulting target range is not valid for the individual methods.
The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} above 2,0. Therefore the evaluation of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then below 2,0.*
The evaluation of method RS-F showed a low variability of results. The quotient S/σ_{pt} was below 2,0. The robust standard deviation is in the lower 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 24% and 31% of the spiking

level of almond to sample B and thus below the range of the recommendations for the applied methods (s. 3.4.3 and p.42 "Recovery rates ELISA for Almond").

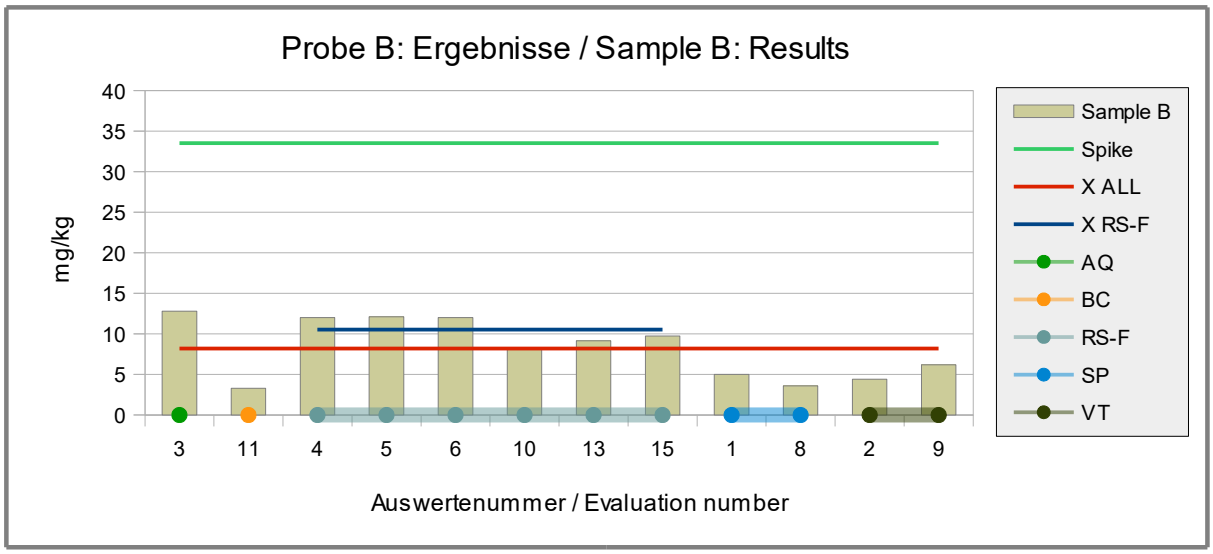


Abb./Fig. 10: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

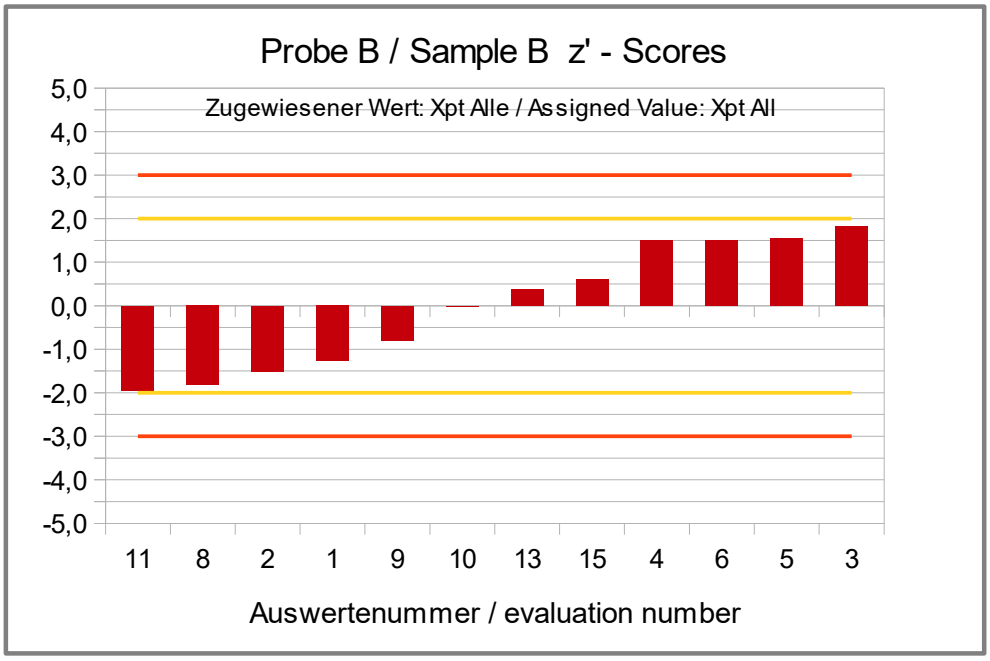


Abb./Fig. 11:
z'-Scores (ELISA Results Almond)
Assigned value robust mean of all results

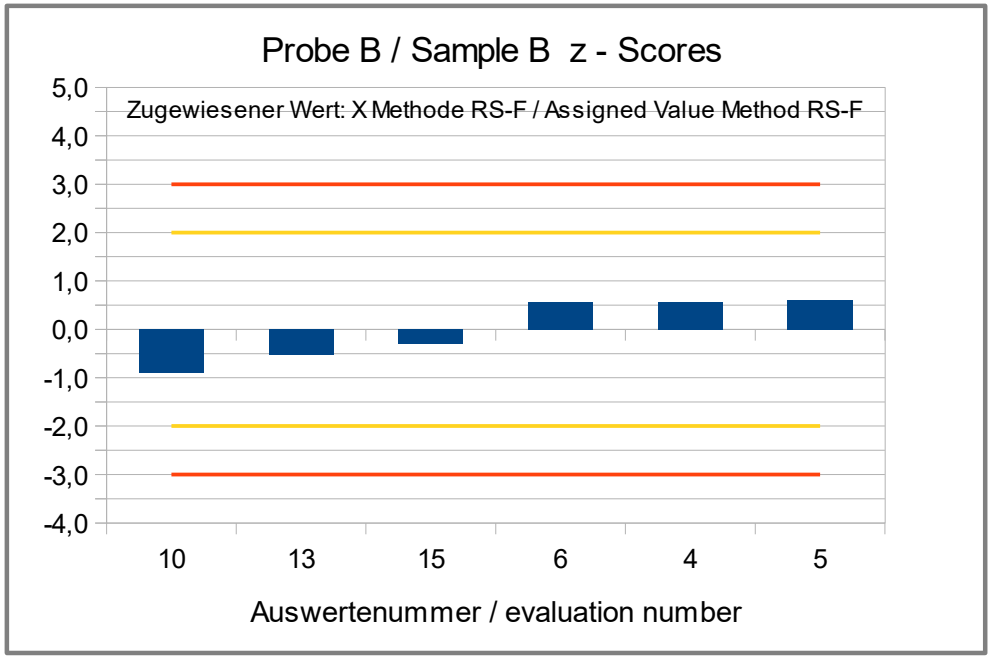


Abb./Fig. 12:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
3	57,3	6,5		AQ	Result converted °
11	9,30	-2,3		BC	
4	23,0	0,23	0,28	RS-F	
5	20,0	-0,32	-0,27	RS-F	
6	20,0	-0,32	-0,27	RS-F	
10	19,8	-0,36	-0,32	RS-F	
13	20,9	-0,15	-0,11	RS-F	
15	29,5	1,4	1,5	RS-F	
1	25,0	0,60		SP	
8	13,0	-1,6		SP	
2	25,9	0,77		VT	
9	20,2	-0,28		VT	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

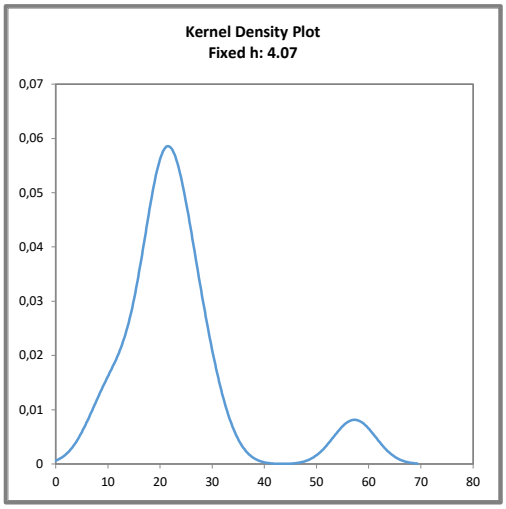


Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a slight shoulder < 10 mg/kg and a side peak at 57 mg/kg, due to a single value outside the target range (method AQ).

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	6
Number of outliers	-	-
Mean	23,7	22,2
Median	20,6	20,5
Robust Mean (X_{pt})	**21,7**	**21,5**
Robust standard deviation (S*)	**6,65**	**2,45**
Target range:		
Target standard deviation σ_{pt}	**5,43**	**5,37**
lower limit of target range	**10,9**	**10,7**
upper limit of target range	**32,6**	**32,2**
Quotient S/σ_{pt}*	*1,2*	*0,46*
Standard uncertainty $U_{(Xpt)}$	*2,40*	*1,25*
Results in the target range	*10*	*6*
Percent in the target range	*83*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation shows nearly a symmetrical distribution of results (one high single value).

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

The robust means of the evaluations were 108% and 106% of the spiking level of almond to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and p.42 "Recovery rates ELISA for Almond").

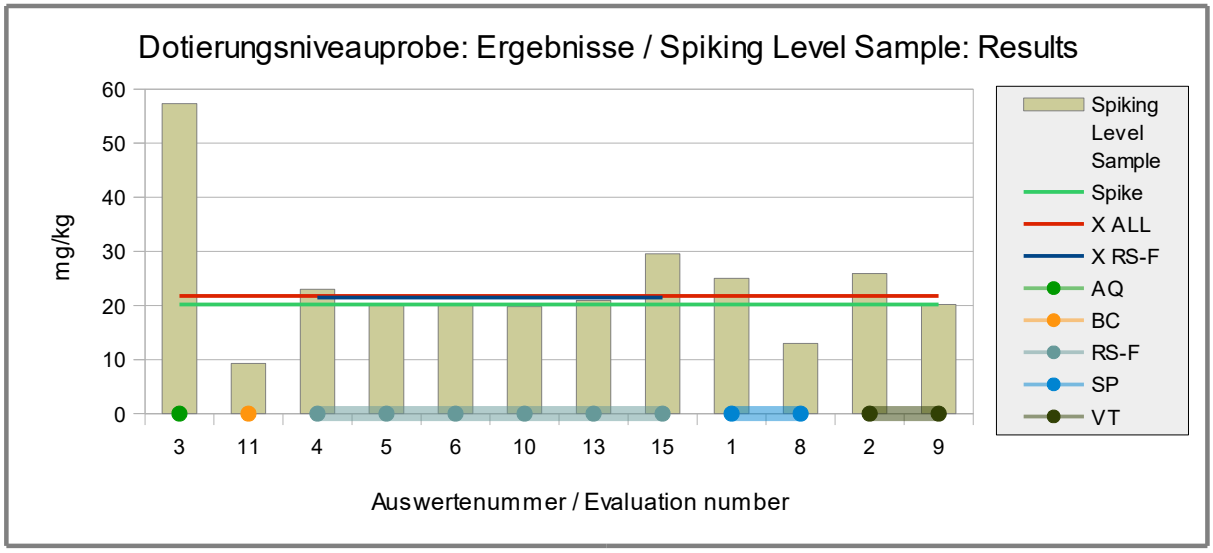


Abb./Fig. 14: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

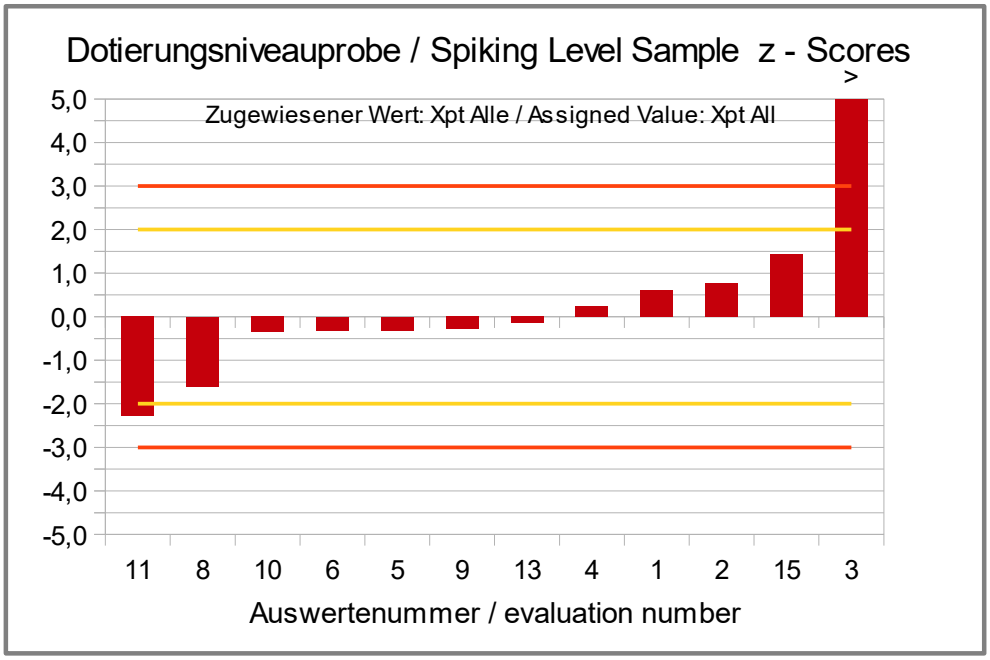


Abb./Fig. 15:
z-Scores (ELISA Results Almond)
Assigned value robust mean of all results

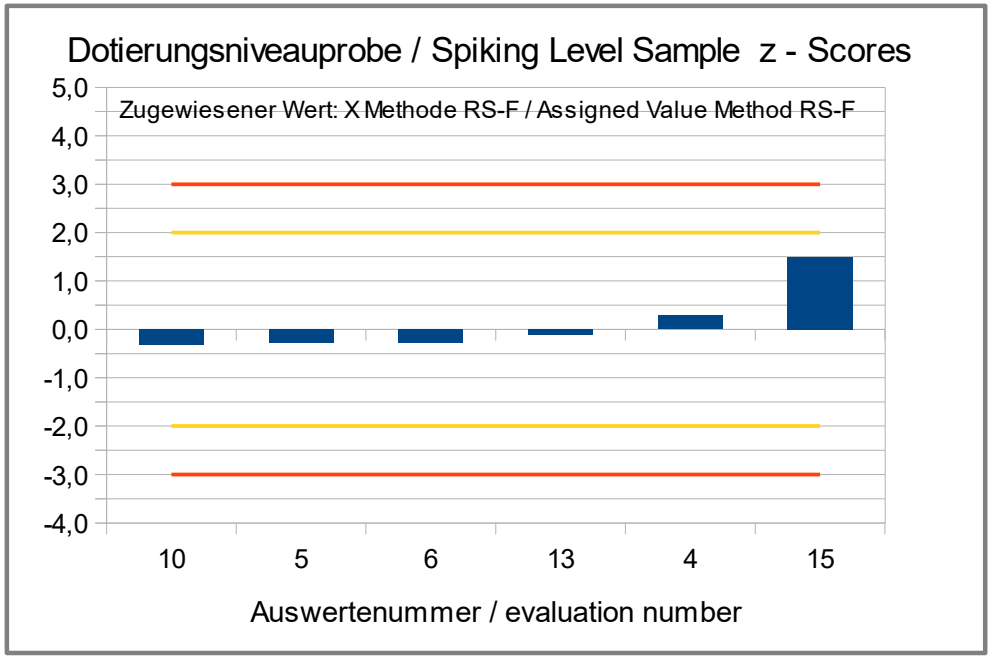


Abb./Fig. 16:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

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}]		
3	57,3	284	7,3	12,8	38	-2,5	AQ	Result converted °
11	9,30	46	-2,2	3,30	10	-3,6	BC	
4	23,0	**114**	0,55	12,0	36	-2,6	RS-F	
5	20,0	**99**	-0,04	12,1	36	-2,6	RS-F	
6	20,0	**99**	-0,04	12,0	36	-2,6	RS-F	
10	19,8	**98**	-0,08	8,16	24	-3,0	RS-F	
13	20,9	**104**	0,14	9,15	27	-2,9	RS-F	
15	29,5	**146**	1,8	9,72	29	-2,8	RS-F	
1	25,0	**124**	0,95	5,00	15	-3,4	SP	
8	13,0	**64**	-1,4	3,60	11	-3,6	SP	
2	25,9	**128**	1,1	4,40	13	-3,5	VT	
9	20,2	**100**	0,00	6,20	19	-3,3	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**10**	Number in RA	**0**
Percent in RA	**83**	Percent in RA	**0**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

83% (13) of the 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 processed, spiked food matrix sample B none of the recovery rates were in the range of acceptance, but all below.
The related z-scores are based on the target standard deviation of 25%.

4.3.2 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		
7	negative		negative		1/2 (50%)	SFA	no positive sample detected
15	negative		positive		2/2 (100%)	SFA	
3	negative		negative		1/2 (50%)	div	no positive sample detected

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
The negative consensus value for sample A was in qualitative agreement with the spiking of sample B.
One negative result was obtained for sample B, therefore no consensus value of ≥75% could be determined.

Qualitative valuation PCR: Spiking Level Sample

Evaluation number	Almond	Almond	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positive			SFA	
15	positive			SFA	
3	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
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative valuation PCR: Sample B and Spiking Level Sample

No quantitative evaluation was done, because there were no quantitative results.

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 Peanut: **Xpt** (Method: RS-F)		ELISA Almond: **Xpt** (div. Methods)		ELISA Almond: **Xpt** (Method: RS-F)	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B°	Sp. Level Sample	Sample B	Sp. Level Sample
1	1,3	0,45	-	-	-1,3	0,60	-	-
2 / 2a	-1,5	-0,98	-	-	-1,5	0,77	-	-
2b	-0,24	1,2	-	-	-	-	-	-
3	-0,24	-0,06	-0,64	-0,41	1,8	6,5	-	-
4	1,1	0,14	0,53	-0,23	1,5	0,23	0,56	0,28
5	0,82	-0,45	0,31	-0,76	1,5	-0,32	0,60	-0,27
6	0,82	0,61	0,31	0,20	1,5	-0,32	0,56	-0,27
7	-	-	-	-	-	-	-	-
8	-0,78	-1,3	-	-	-1,8	-1,6	-	-
9	-	-	-	-	-0,79	-0,28	-	-
10	-0,56	-0,39	-0,92	-0,71	-0,02	-0,36	-0,90	-0,32
11	-0,82	-0,83	-	-	-1,9	-2,3	-	-
12	-2,9	-3,3	-	-	-	-	-	-
13	1,1	1,5	0,58	1,0	0,38	-0,15	-0,52	-0,11
14	0,63	0,68	0,14	0,26	-	-	-	-
15	0,11	1,1	-0,33	0,62	0,60	1,4	-0,30	1,5

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

*z-Scores

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)

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

Evaluation number	ELISA Peanut:		ELISA Almond:	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
1	-1,1	6,5	-3,4	0,95
2 / 2a	-2,7	3,1	-3,5	1,1
2b	-1,9	8,2		
3	-1,9	5,3	-2,5	7,3
4	-1,2	5,8	-2,6	0,55
5	-1,4	4,4	-2,6	-0,04
6	-1,4	6,9	-2,6	-0,04
7				
8	-2,2	2,4	-3,6	-1,4
9			-3,3	0,00
10	-2,1	4,5	-3,0	-0,08
11	-2,3	3,5	-3,6	-2,2
12	-3,4	-2,3		
13	-1,2	9,1	-2,9	0,14
14	-1,5	7,0		
15	-1,8	8,0	-2,8	1,8

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)

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
BC	11	28.09.20	negative	<1	positive	13,2	positive	39,9	1	1	50	Whole Peanut	BioCheck ELISA Peanut-Check
BK	2a	05.10.20	negative	<1	positive	10,2	positive	38		1		Peanut	BioKits Peanut Assay Kit, Neogen
MI-II	8	16.09.20	negative	<0,2	positive	3,1	positive	7,9	0,2	0,2		Peanutprotein	Peanut ELISA Kit-II, Morinaga
RS-F	3		negative		positive	15,6	positive	49,6	2,5	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	4	23.09.20	negative		positive	21	positive	52	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	5	22.10.20	negative	< 2.5	positive	20	positive	44,7	1,5	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	6	20.10.20	negative	-	positive	20	positive	58	0,3	1		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	10	28.09.20	negative	<2,5	positive	14,29	positive	45,39	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	13	04.11.20	negative	<2,5	positive	21,26	positive	69,58	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	16.10.20	negative		positive	19,2	positive	58,8	0,13	2,5	18,5	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	15	06.11.20	negative	<1	positive	17,04	positive	63,85	1	1	31,4	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
SP	1	15.09.20	negative	0	positive	22	positive	56	0.1	1		Peanut	Eurofins SensiSpec Peanut ELISA Kit
VT	2b	15.10.20	negative	<2,5	positive	15,6	positive	64,8		2,5		Peanut	Veratox Peanut, Neogen
VT	12		negative		positive	4,39	positive	8,87		2,5		Peanut	Veratox Peanut, Neogen

* 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 accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BC	11		0.5g sample, 10ml Extraction buffer, 15mins at 60C	YES	
BK	2a		as stipulated in kit insert	yes	
MI-II	8	detects peanut proteins	as per Kit instructions	yes	M2120 Peanut Sensitive ELISA Kit II Morinaga
RS-F	3				
RS-F	4		10 min at 60°C shaking water bath	yes	
RS-F	5				
RS-F	6			yes	
RS-F	10		as per Kit instructions	yes	
RS-F	13	antibodies specifically detect peanut proteins, including Ara h 1 and Ara h 2	Extraction: with Allergen extraction buffer, 10 min., 60 °C	yes	
RS-F	14	Arah1 e Arah2	Ridascreen extraction buffer	YES	
RS-F	15	As per kit instructions	As per Kit instructions	Yes	
SP	1				
VT	2b		as stipulated in kit insert	yes	
VT	12				

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	3		negative		positive	2,7	positive	12,1	0,4	0,4		Almondprotein	AgraQuant ELISA Almond COKAL0748, RomerLabs
BC	11	28.09.20	negative	<0.5	positive	3,3	positive	9,3	0,5	0,5	50	Whole Almond	BioCheck ELISA Almond-Check
RS-F	4	23.09.20	negative		positive	12	positive	23	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	5	22.10.20	negative	< 2.5	positive	12,1	positive	20	1,5	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	6	20.10.20	negative	-	positive	12	positive	20	0,3	1		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	10	13.10.20	negative	<2,5	positive	8,16	positive	19,78	0,23	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	13	04.11.20	negative	<2,5	positive	9,15	positive	20,91	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	15	06.11.20	negative	<2.5	positive	9,72	positive	29,54	2,5	2,5	26,48	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
SP	1	15.09.20	negative	0	positive	5	positive	25	0.2	0.4		Almond	Eurofins SensiSpec Almond ELISA Kit
SP	8	18.09.20	negative	<0,4	positive	3,6	positive	13	0,4	0,4		Almond	Eurofins SensiSpec Almond ELISA Kit
VT	2	15.10.20	negative	<2,5	positive	4,4	positive	25,9		2,5		Almond	Veratox Almond, Neogen
VT	9		0,2		6,2		20,2					Please select!	Selection Almond-Kits:

* NWG Nachweisgrenze / 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 accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	3				
BC	11		0.5g sample, 10ml Extraction buffer, 15mins at 60C	YES	
RS-F	4		10 min at 60°C shaking water bath	yes	
RS-F	5			yes	
RS-F	6			yes	
RS-F	10		as per Kit instructions	yes	
RS-F	13	antibodies specifically detect proteins from almonds	Extraction: with Allergen extraction buffer, 10 min., 60 °C	yes	
RS-F	15	As per kit instructions	As per Kit instructions	Yes	
SP	1				
SP	8	detects almond proteins	as per Kit instructions	yes	
VT	2		as stipulated in kit insert	yes	
VT	9		15 min / 60°C	no	

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
SFA	7		negative		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	06.11.20	negative		positive		positive		1			Peanut	Sure Food ALLERGEN, R-Biopharm / Congen
div	3		negative		positive		positive					Please select!	Selection PCR-Methods
div	8	17.09.20	negative		positive		positive		5			Peanut DNA	other: please fill in!

* 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	
SFA	7				
SFA	15	As per kit instructions	As per Kit instructions	Yes	
div	3				
div	8		CTAB / Proteinas K / Rnase A / Promega Maxwell / Real-time PCR / 45 Cycles	yes	internal method

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
SFA	7		negative		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	06.11.20	negative		positive		positive		1			Almond	Sure Food ALLERGEN, R-Biopharm / Congen
div	3		negative		negative		positive					Almond DNA	foodproof Detection Kit, BIOTECON Diagnostics

* 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	
SFA	7				
SFA	15	As per kit instructions	As per Kit instructions	No	
div	3				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL05 Sample B

Weight whole sample	2,96	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	40	16,0
2	5,01	46	18,4
3	5,03	43	17,1
4	5,02	45	17,9
5	5,01	50	20,0
6	4,97	51	20,5
7	5,00	47	18,8
8	5,00	52	20,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	46,8	Particles
Standard deviation	4,20	Particles
χ^2 (CHI-Quadrat)	2,64	
Probability	**92**	%
Recovery rate	88	%

Normal distribution		
Number of samples	8	
Mean	18,7	mg/kg
Standard deviation	1,68	mg/kg
rel. Standard deviaton	9,0	%
Horwitz standard deviation	10,3	%
HorRat-value	**0,87**	
Recovery rate	88	%

Microtracer Homogeneity Test
DLA ptAL05 Spiking Level Sample

Weight whole sample	1,55	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	58	23,2
2	4,98	53	21,3
3	5,00	51	20,4
4	5,00	50	20,0
5	4,97	56	22,5
6	4,98	49	19,7
7	4,98	49	19,7
8	5,04	56	22,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	52,7	Particles
Standard deviation	3,49	Particles
χ^2 (CHI-Quadrat)	1,62	
Probability	**98**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	21,1	mg/kg
Standard deviation	1,40	mg/kg
rel. Standard deviaton	6,6	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,65**	
Recovery rate	107	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL05 - 2020***
PT name	***Allergens V: Peanut and Almond in Pastry with "Spiking Level Sample“***
Sample matrix (processing)	***Samples A + B:*** *Cocoa biscuits (baked at 150 ° C) / Ingredients: wheat flour, sugar, palm oil, glucose syrup, low-fat cocoa powder, raising agents: sodium carbonates, diphosphates; salt, apple extract, skimmed milk powder, emulsifier: lecithins (soy); flavors, acidulants: citric acid; starch, whole egg powder as well as butter, eggs, other additives and allergenic foods (one of the two 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 (Peanut protein, DNA), Almond (Almond protein, DNA) Samples A + B: < 500 mg/kg Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest November 06th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
ALS Life Sciences Division, Food and Pharmaceutical	CHATTERIS, CAMBRIDGESHIRE	GREAT BRITAIN
		ITALY
		SWITZERLAND
		CANADA
		Germany
		Germany
		SWITZERLAND
		Germany
		FRANCE
		Germany
		Germany
		SWITZERLAND
		HUNGARY
		GREAT BRITAIN
		CROATIA

[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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