Evaluation Report

proficiency test

DLA ptAL08 (2020)

Allergens VIII:

Almond and Cashew

in Veggie Burger Powder

DLA - Proficiency Tests GmbH
Hauptstr. 80
23845 Oering/Germany

proficiency-testing@dla-lvu.de *www.dla-lvu.de*

Coordinator of this PT:
Matthias Besler-Scharf, Ph.D.

1st Correction 22/05/2021:

The total protein content of cashew was corrected in table 1 composition of DLA samples (page 5). The content is 18,5%, not 21,1%.
The evaluation of participants' results has not been affected.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Hauptstr. 80, 23845 Oering, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptAL08 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (22 May 2021) 1. Korrektur / 1st Correction 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: 22 May 2021
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 of the food matrix samples is a common in commerce veggie burger mixture (powder). The basic composition of samples A and B was the same (see table 1). After crushing and sieving (mesh 1,5 mm) the basic mixture was homogenized.
Afterwards the **spiked samples A** were produced as follows:
The spiking materials containing the allergenic ingredients almond and cashew were milled and homogenized, then added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 3 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 and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Probe A**	**Probe B**	**Dotierungs-niveauprobe**
Veggie burger powder Ingredients: Whole oat flakes, wholegrain spelled semolina, wholegrain spelled flakes, corn flakes (corn flour, raw cane sugar, sea salt), onions, leeks, carrots, sunflower oil, sea salt, tomato powder, zucchini, garlic, spices (pepper, mace, turmeric), herbs (lovage leaves, oregano) Nutrients per 100 g: Fat 6,2 g, Carbohydrates 57 g, Protein 12 g, Salt 2,1 g	99,86 g/100 g	100 g/100g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,8 g/100 g
Almond, roasted milled, mixture (23 products from USA, Europe, Australia, Middle East) - as almond* - *thereof 21,1% total protein***	 19,2 mg/kg 4,06 mg/kg	-	 18,5 mg/kg 3,91 mg/kg
Cashew, roasted milled, mixture (9 products from Asia and unknown origin) - as cashew* - *thereof 18,5% total protein***	 10,1 mg/kg 1,86 mg/kg	-	 12,3 mg/kg 2,28 mg/kg
further Ingredients: Maltodextrin and silicon dioxide	<0,2 g/100 g	-	<0,2 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=5,18 for almond protein and with F=5,30 for cashew protein)

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 A and the spiking level sample showed a probability of 68% and 100%. 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 1,3 and 0,42. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

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 A by all ELISA tests for almond (Immunolab and AgraQuant) and cashew (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].

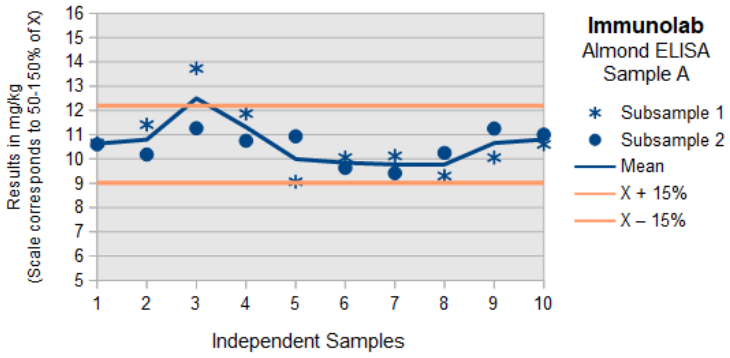
ELISA-Tests: Homogenität Mandel / Homogeneity Almond

Immunolab Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 10,6 ± 0,73 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	10,7	10,6	10,6
2	11,4	10,2	10,8
3	13,7	11,3	12,5
4	11,9	10,8	11,3
5	9,06	10,9	10,0
6	10,1	9,63	9,85
7	10,1	9,42	9,77
8	9,30	10,2	9,78
9	10,1	11,3	10,7
10	10,6	11,0	10,8

General average X	10,6	
SD of sample means Sx	0,85	8,0%
SD within-samples Sw	0,62	5,9%
SD between-samples Ss	0,73	6,8%

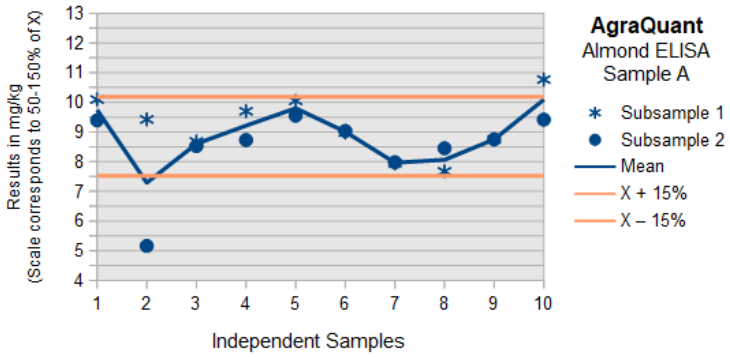


AgraQuant Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 8,85 ± 0,73 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	10,1	9,39	9,74
2	9,42	5,16	7,29
3	8,68	8,53	8,60
4	9,70	8,73	9,21
5	10,0	9,55	9,80
6	8,98	9,04	9,01
7	7,95	7,98	7,97
8	7,67	8,45	8,06
9	8,75	8,75	8,75
10	10,8	9,42	10,1

General average X	8,85	
SD of sample means Sx	0,90	10,2%
SD within-samples Sw	0,75	8,4%
SD between-samples Ss	0,73	8,2%



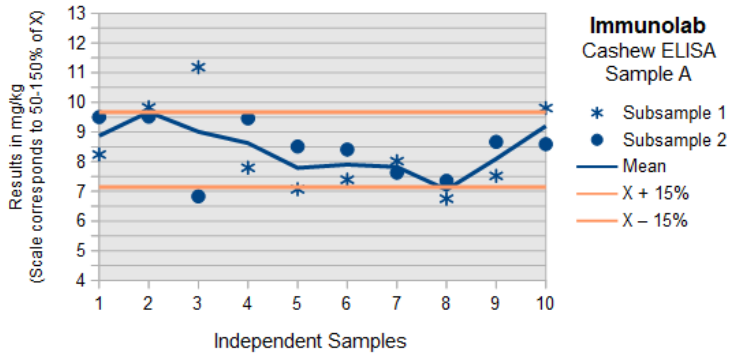
ELISA-Tests: Homogenität Cashew / Homogeneity Cashew

Immunolab Cashew ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Cashew 8,40 ± 0,52 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	8,24	9,50	8,87
2	9,83	9,52	9,67
3	11,18	6,83	9,01
4	7,80	9,46	8,63
5	7,07	8,51	7,79
6	7,39	8,41	7,90
7	8,02	7,63	7,82
8	6,75	7,36	7,05
9	7,52	8,67	8,09
10	9,81	8,59	9,20

General average X	8,40	
SD of sample means Sx	0,800	9,5%
SD within-samples Sw	0,863	10,3%
SD between-samples Ss	0,517	6,2%

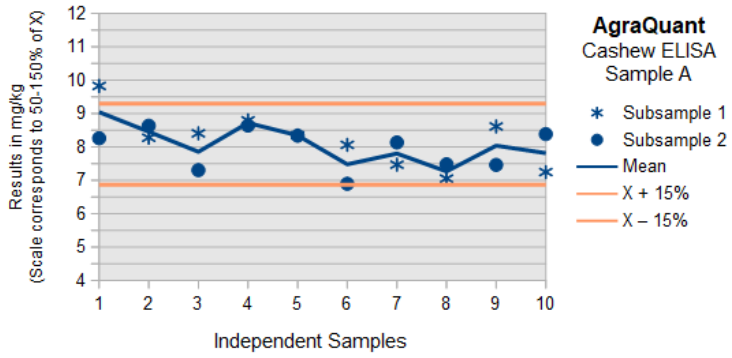


AgraQuant Cashew ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Cashew 8,08 ± 0,45 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	9,82	8,26	9,04
2	8,27	8,63	8,45
3	8,40	7,30	7,85
4	8,80	8,64	8,72
5	8,35	8,34	8,34
6	8,06	6,89	7,48
7	7,47	8,13	7,80
8	7,05	7,48	7,26
9	8,61	7,46	8,04
10	7,25	8,38	7,81

General average X	8,08	
SD of sample means Sx	0,55	6,9%
SD within-samples Sw	0,46	5,7%
SD between-samples Ss	0,45	5,6%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,37 (18°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 49nd week of 2020. The testing method was optional. The tests should be finished at 12th February 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 Almond and Cashew in the range of mg/kg in the matrix of Veggie Burger Powder. 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 5.4 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 22 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^x_{ALL}
ii) ***Robust standard deviation of single methods*** - $S^x_{METHOD\ i}$
with at least 5 quantitative results given.

3.3 Exclusion of results and outliers

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

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 25 – 42% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

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} [33-35]

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

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of 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).

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 **almond protein** or **cashew protein** were converted by DLA to **total food items (almond, cashew)** 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 Almond

4.1.1 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		
7	positive	9,15	negative		2/2 (100%)	AQ	
2	positive	35,1	negative	0,95	2/2 (100%)	IL	Result converted*
8	positive	11,0	negative	<1	2/2 (100%)	NL	
13	positive	8,30	negative	<0,2	2/2 (100%)	NL	
1	positive	16,3	negative	<0,1	2/2 (100%)	RS-F	
3	positive	17,8	negative	<2,5	2/2 (100%)	RS-F	
6	positive	16,6	negative	<2,5	2/2 (100%)	RS-F	
10	positive	17,0	negative	<2,5	2/2 (100%)	RS-F	
14	positive	18,0	negative	<2,5	2/2 (100%)	RS-F	
15	positive	13,0	negative	<2,50	2/2 (100%)	RS-F	
16	positive	17,8	negative		2/2 (100%)	RS-F	
18	positive	19,0	negative	<2,5	2/2 (100%)	RS-F	
19	positive		negative		2/2 (100%)	RS-F	
21	positive	16,9	negative	<2,50	2/2 (100%)	RS-F	
22	positive	23,3	negative	<2.5	2/2 (100%)	RS-F	
4	positive	12,0	negative	0	2/2 (100%)	SP	
11	positive	9,30	negative	<0,4	2/2 (100%)	SP	
5	positive	9,16	negative	<2,5	2/2 (100%)	VT	
17	positive	8,20	negative	<2,5	2/2 (100%)	VT	
20	positive	9,00	negative		2/2 (100%)	VT	

* Calculation see p. 19

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

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = nutriLinia® Allergen-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 A.

Quantitative valuation of ELISA results: Sample A

Evaluation number	Almond	z-Score Xpt_{Peak10}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
7	9,15	-0,13		AQ	
2	35,1			IL	Result converted *
8	11,0	0,66		NL	
13	8,30	-0,48		NL	
1	16,3		-0,26	RS-F	
3	17,8		0,09	RS-F	
6	16,6		-0,19	RS-F	
10	17,0		-0,10	RS-F	
14	18,0		0,13	RS-F	
15	13,0		-1,0	RS-F	
16	17,8		0,09	RS-F	
18	19,0		0,36	RS-F	
21	16,9		-0,12	RS-F	
22	23,3		1,4	RS-F	
4	12,0	1,1		SP	
11	9,30	-0,06		SP	
5	9,16	-0,12		VT	
17	8,20	-0,53		VT	
20	9,00	-0,19		VT	

* Calculation p. 19

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

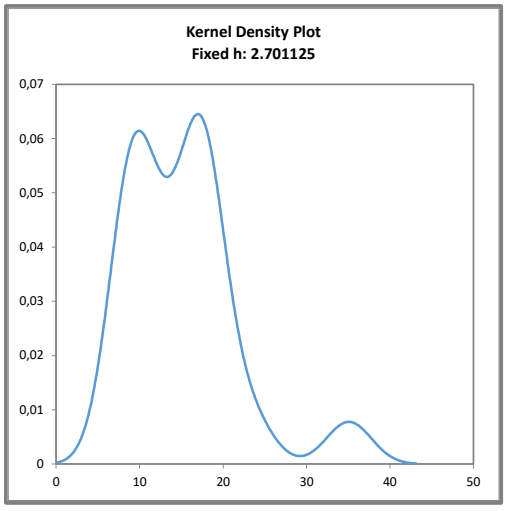


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimate shows a bimodal distribution of the results with a maximum at approx. 17 mg/kg due to results of method RS-F and a maximum at approx. 10 mg/kg due to the other methods.

Characteristics: Quantitative evaluation ELISA Almond

Sample A

Statistic Data	**Results Peak10* [mg/kg]**	**Method RS-F**** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{Peak10}}$	$X_{pt_{METHODE\ RS\text{-}F}}$
Number of results	8	10
Number of outliers	0	0
Mean	9,51	17,6
Median	9,15	17,4
Robust Mean (X)	**9,44**	**17,4**
Robust standard deviation (S*)	**1,33**	**1,48**
Target range:		
Target standard deviation σ_{pt}	**2,36**	**4,36**
lower limit of target range	**4,72**	**8,71**
upper limit of target range	**14,2**	**26,1**
Quotient S/σ_{pt}*	*0,56*	*0,34*
Standard uncertainty $U_{(Xpt)}$	*0,586*	*0,585*
Results in the target range	*8*	*10*
Percent in the target range	*100*	*100*

Method:
***** „Peak10": AQ = AgraQuant, RomerLabs, NL = NutriLinia, SP = SensiSpec ELISA Kit, Eurofins, VT = Veratox, Neogen
** RS-F = Ridascreen® Fast, R-Biopharm

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a bimodal distribution of the results. The peak at approx. 17 mg/kg is cause by results of method RS-F method. A joint evaluation of the results of all methods was therefore not carried out. The other methods, which showed a peak at approx. 10 mg/kg, were evaluated separately.

The evaluation of "peak 10" and the evaluation of results from method RS-F showed a low variability of results, with quotients S/σ_{pt} below 1,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 49% and 91% of the spiking level of almond to sample A and thus slightly below and within the range of the recommendations for the applied methods (s. 3.4.3 and p.31 "Recovery rates ELISA for Almond").

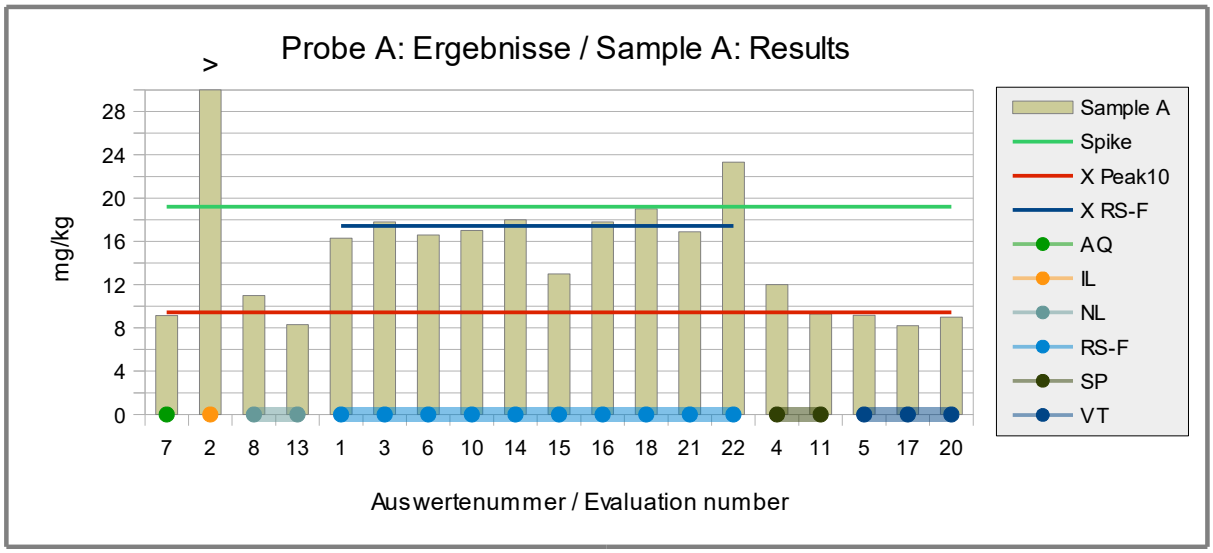


Abb./Fig. 2: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

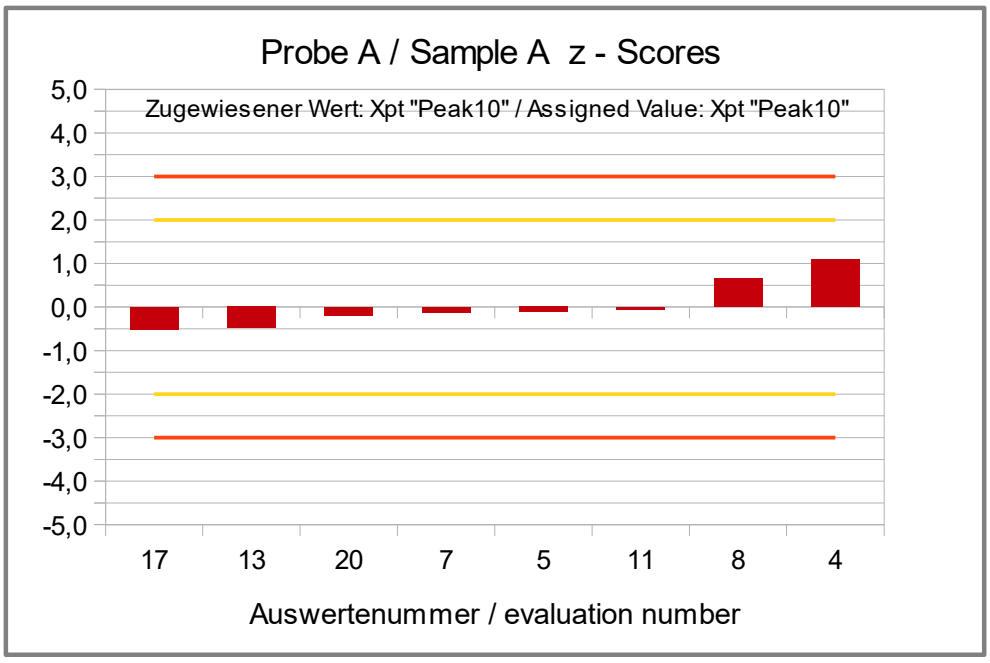


Abb./Fig. 3:
z-Scores (ELISA Results Almond)
Assigned value robust mean of all results of "peak 10"

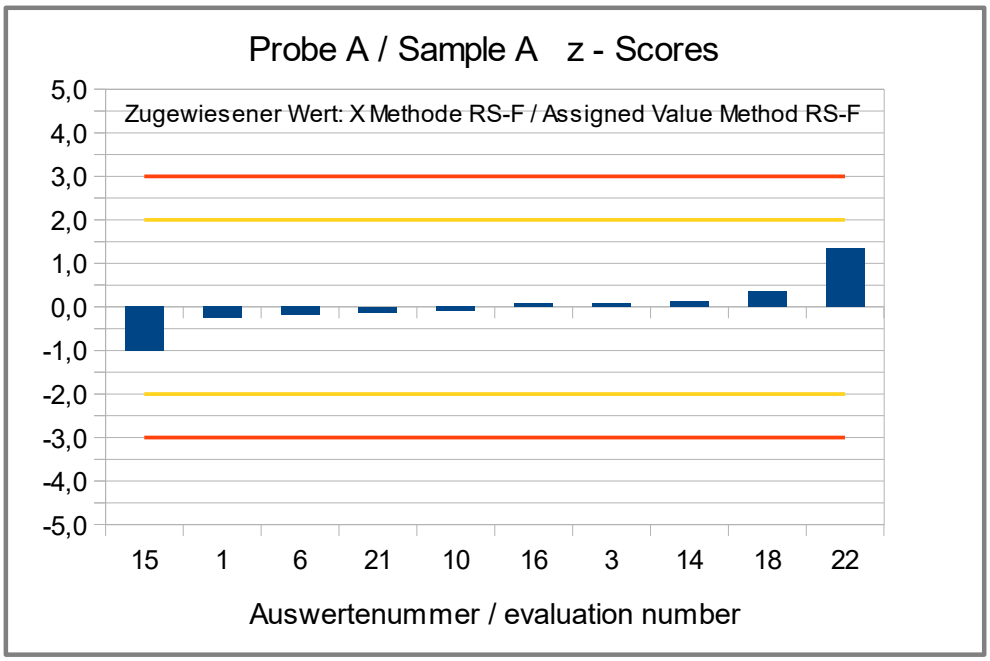


Abb./Fig. 4:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
7	12,7	-1,0		AQ	
2	52,1	8,3		IL	Result converted °
8	12,0	-1,2		NL	
13	13,0	-0,94		NL	
1	21,0	0,95	0,45	RS-F	
3	21,0	0,95	0,45	RS-F	
6	17,6	0,14	-0,27	RS-F	
10	18,6	0,38	-0,06	RS-F	
14	17,0	0,01	-0,39	RS-F	
15	14,0	-0,70	-1,03	RS-F	
16	21,6	1,1	0,58	RS-F	
18	>20,0			RS-F	
21	16,2	-0,19	-0,57	RS-F	
22	22,8	1,4	0,83	RS-F	
4	14,0	-0,70		SP	
11	12,0	-1,2		SP	
5	16,2	-0,19		VT	
17	17,5	0,12		VT	
20	15,1	-0,44		VT	

° Calculation see p.19

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

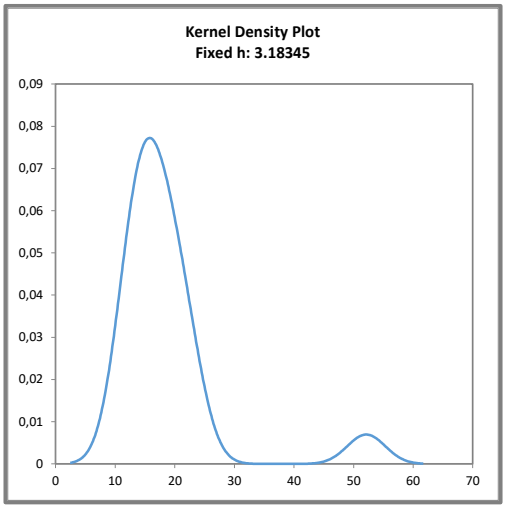


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 side peak at 52 mg/kg, due to a single value outside the target range (method IL).

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	18	9
Number of outliers	0	0
Mean	18,6	18,9
Median	16,6	18,6
Robust Mean (X)	**17,0**	**18,9**
Robust standard deviation (S*)	**4,23**	**3,31**
Target range:		
Target standard deviation σ_{pt}	**4,24**	**4,72**
lower limit of target range	**8,49**	**9,43**
upper limit of target range	**25,5**	**28,3**
Quotient S/σ_{pt}*	*1,0*	*0,70*
Standard uncertainty $U_{(Xpt)}$	*1,25*	*1,38*
Results in the target range	*17*	*9*
Percent in the target range	*94*	*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 to low variability of results. 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 92% and 102% 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").

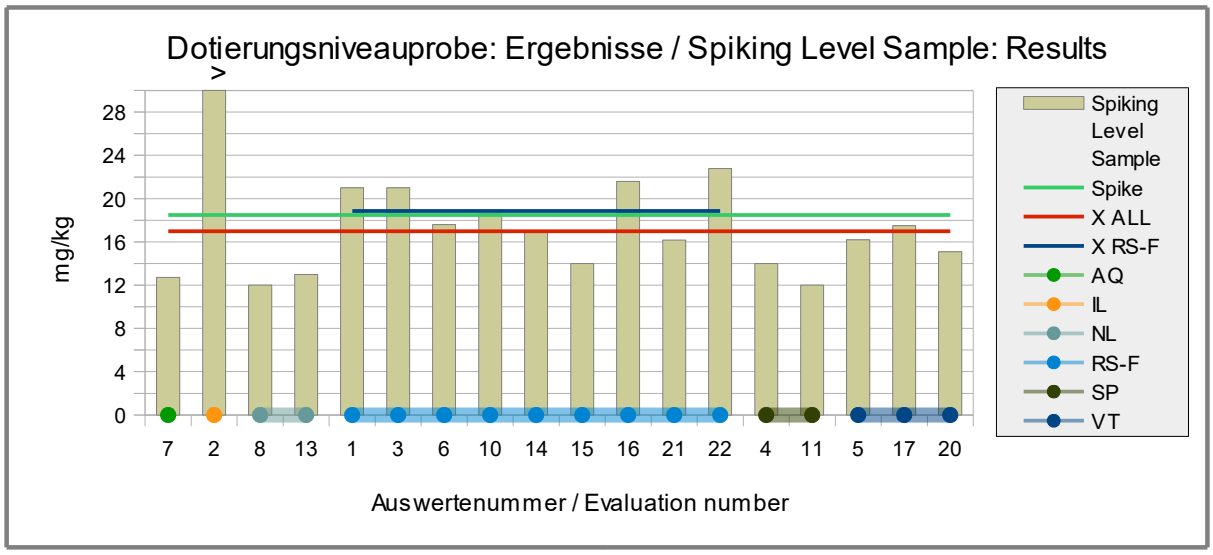


Abb./Fig. 6: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

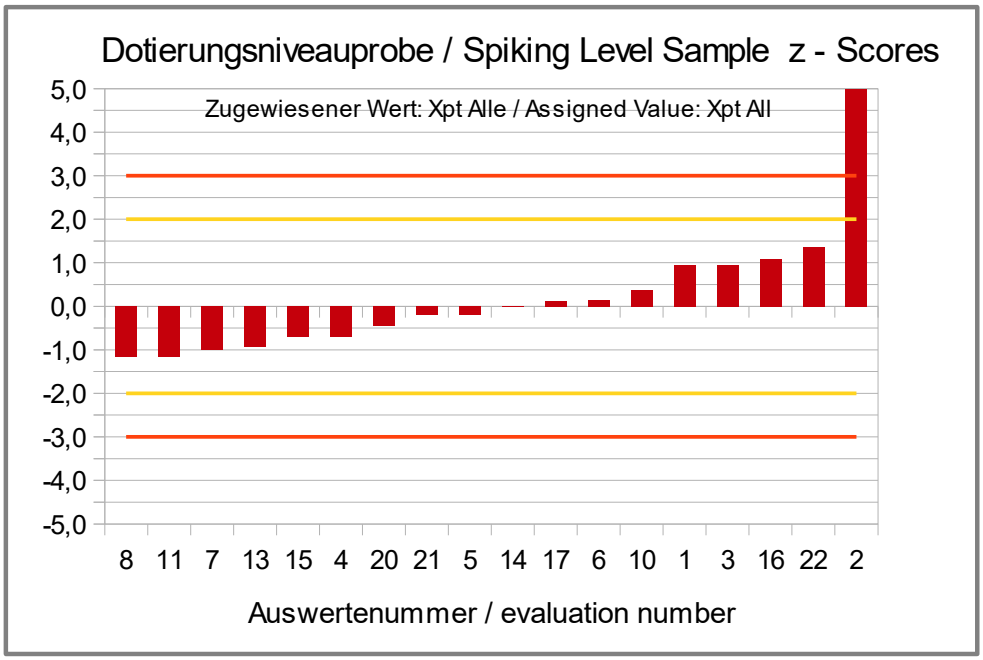


Abb./Fig. 7:
z-Scores (ELISA Results Almond)
Assigned value robust mean of all results

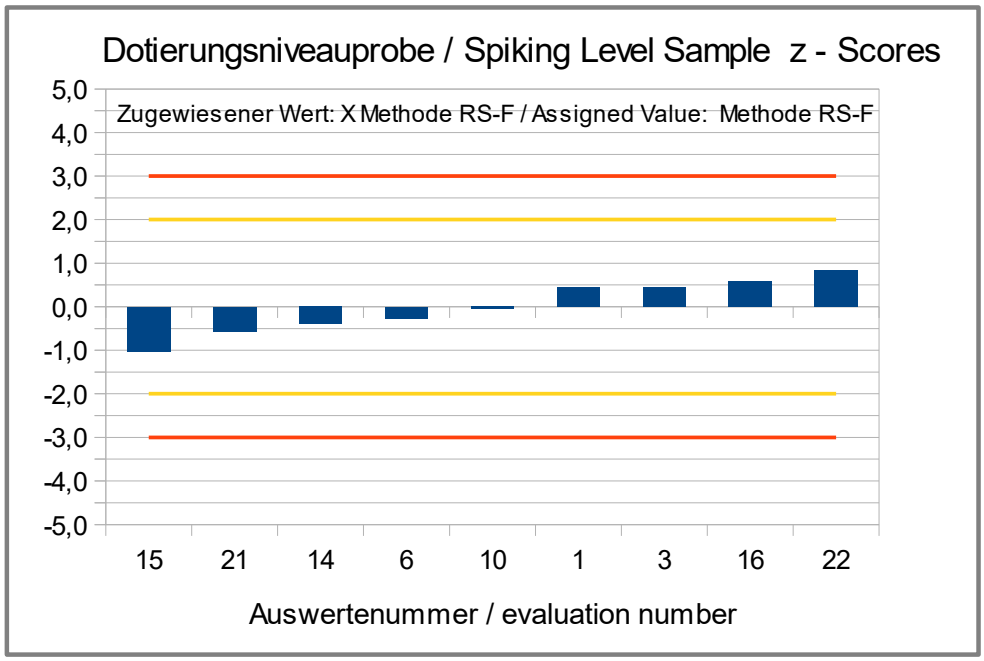


Abb./Fig. 8:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
7	12,7	**69**	-1,2	9,15	48	-2,1	AQ	
2	52,1	282	7,3	35,1	183	3,3	IL	Result converted °
8	12,0	**65**	-1,4	11,0	**57**	-1,7	NL	
13	13,0	**70**	-1,2	8,30	43	-2,3	NL	
1	21,0	**114**	0,54	16,3	**85**	-0,61	RS-F	
3	21,0	**114**	0,54	17,8	**93**	-0,29	RS-F	
6	17,6	**95**	-0,19	16,6	**86**	-0,54	RS-F	
10	18,6	**101**	0,02	17,0	**88**	-0,46	RS-F	
14	17,0	**92**	-0,32	18,0	**94**	-0,25	RS-F	
15	14,0	**76**	-0,97	13,0	**68**	-1,29	RS-F	
16	21,6	**117**	0,67	17,8	**93**	-0,29	RS-F	
21	16,2	**88**	-0,50	16,9	**88**	-0,49	RS-F	
22	22,8	**123**	0,93	23,3	**121**	0,86	RS-F	
4	14,0	**76**	-0,97	12,0	**62**	-1,5	SP	
11	12,0	**65**	-1,4	9,30	48	-2,1	SP	
5	16,2	**88**	-0,50	9,16	48	-2,1	VT	
17	17,5	**95**	-0,21	8,20	43	-2,3	VT	
20	15,1	**82**	-0,73	9,00	47	-2,1	VT	

° Calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**17**	Number in RA	**11**
Percent in RA	**94**	Percent in RA	**61**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

94% (17) 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 spiked food matrix sample A 61% (11) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.1.2 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	positive		negative		2/2 (100%)	SFA	
9	positive		negative		2/2 (100%)	SFA	
16	positive		negative		2/2 (100%)	SFA	
3	negative		negative		1/2 (50%)	div	
13a	positive	<5 (2)	negative		2/2 (100%)	div	
13b	positive	0,360	negative		2/2 (100%)	div	
13c	positive	2,00	negative		2/2 (100%)	div	
19	negative		negative		1/2 (50%)	div	

	Sample A		Sample B	
Number positive	6		0	
Number negative	2		8	
Percent positive	75		0	
Percent negative	25		100	
Consensus value	positive		negative	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode

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

Quantitative Valuation PCR: Sample A

An evaluation of the quantitative results was not carried out because too few results were available

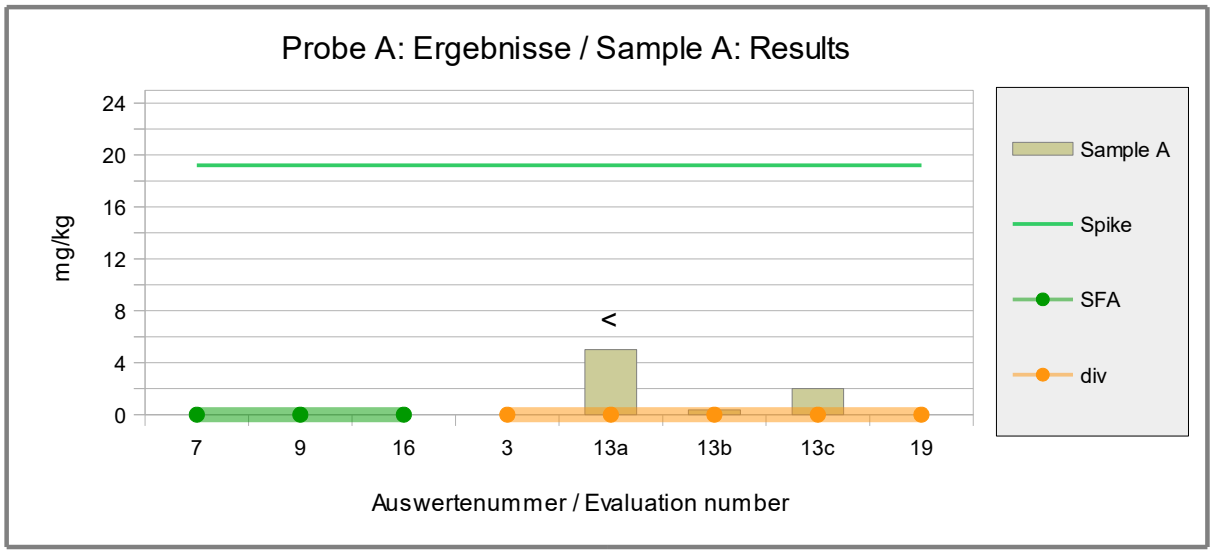


Abb./Fig. 9: PCR Results Almond
green line = Spiking level
round symbols = Applied methods (see legend)

Qualitative valuation PCR: Spiking Level Sample

An evaluation of the quantitative results was not carried out because too few results were available

Evaluation number	Almond	Almond	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positiv			SFA	
9	positiv			SFA	
16	positiv			SFA	
3	positiv			div	
13a	positiv	<5 (1,4)		div	
13b	positiv	0,470		div	
13c	positiv	2,20		div	
19	positiv			div	

Number positive	8	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positiv	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode

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

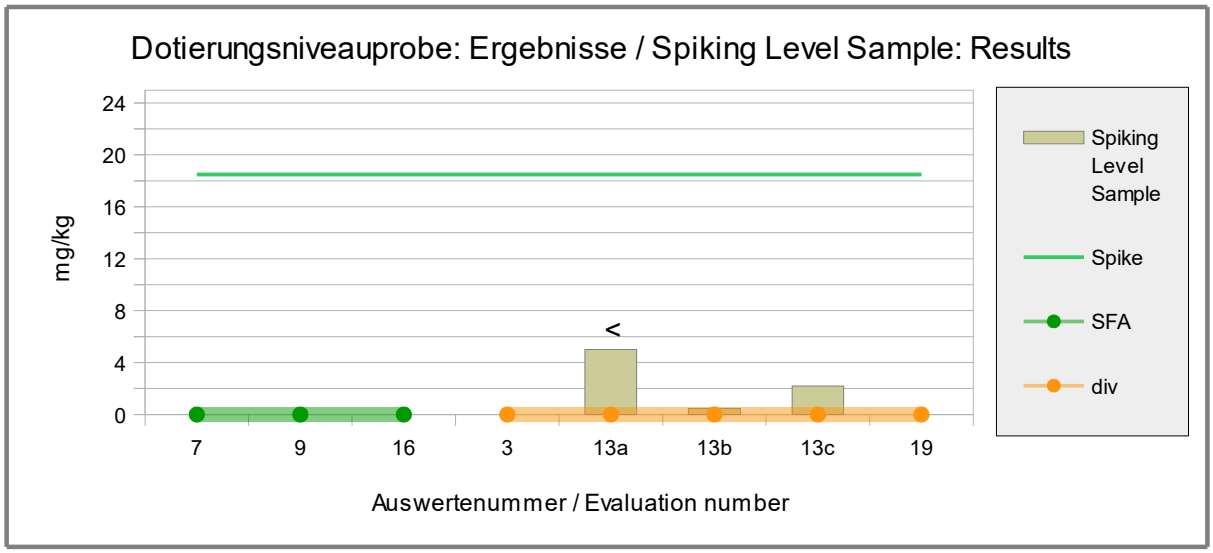


Abb./Fig. 10: PCR Results Almond
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Almond: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
7							SFA	
9							SFA	
16							SFA	
3							div	
13a	<5			<5			div	
13b	0,470	2,5	-3,9	0,360	1,9	-3,9	div	
13c	2,20	12	-3,5	2,00	10	-3,6	div	
19							div	

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:

Two quantitative PCR results were submitted for almond. The recovery rates for the spiking level sample as well as for the spiked food matrix sample A were well below the AOAC requirement of 50-150%.
The related z-scores are based on the target standard deviation of 25%.

4.2 Proficiency Test Cashew

4.2.1 ELISA Results: Cashew

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		
20	positiv	12,3	negativ		2/2 (100%)	3M	
1	positiv	13,4	negativ	<0,2	2/2 (100%)	AQ	
22	positiv	13,7	negativ	<2	2/2 (100%)	BC	
17	positiv	8,90	negativ	<2.0	2/2 (100%)	BF	
21	positiv	11,2	negativ	<2.00	2/2 (100%)	BF	
5	positiv	19,4	negativ	<4,9	2/2 (100%)	ET	Results converted °
3	positiv	4,70	negativ	<2.5	2/2 (100%)	RS-F	
6	positiv	10,1	negativ	<2,5	2/2 (100%)	RS-F	
7	positiv	10,2	negativ		2/2 (100%)	RS-F	
8	positiv	13,0	negativ	<1	2/2 (100%)	RS-F	
10	positiv	8,80	negativ	<2,5	2/2 (100%)	RS-F	
12	positiv	16,2	negativ	-	2/2 (100%)	RS-F	
14	positiv	11,0	negativ	<2.5	2/2 (100%)	RS-F	
15	positiv	8,00	negativ	<2.50	2/2 (100%)	RS-F	
18	positiv	9,73	negativ	<2,5	2/2 (100%)	RS-F	
19	positiv		negativ		2/2 (100%)	RS-F	
4	positiv	11,0	negativ	0	2/2 (100%)	SP	
11	positiv	6,30	negativ	<2	2/2 (100%)	SP	

* Calculation see p. 19

	Sample A		Sample B	
Number positive	18		0	
Number negative	0		18	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positiv		negativ	

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ET = Elution Technologies ELISA Kit
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Cashew	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
20	12,3	0,50		3M	
1	13,4	0,92		AQ	
22	13,7	1,0		BC	
17	8,90	-0,73		BF	
21	11,2	0,10		BF	
5	19,4	3,1		ET	Result converted *
3	4,70	-2,3	-2,1	RS-F	
6	10,1	-0,29	0,00	RS-F	
7	10,2	-0,25	0,04	RS-F	
8	13,0	0,77	1,1	RS-F	
10	8,80	-0,77	-0,52	RS-F	
12	16,2	2,0	2,4	RS-F	
14	11,0	0,04	0,35	RS-F	
15	8,00	-1,1	-0,84	RS-F	
18	9,73	-0,43	-0,15	RS-F	
4	11,0	0,04		SP	
11	6,30	-1,7		SP	

* Calculation see p. 19

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ET = Elution Technologies ELISA Kit
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

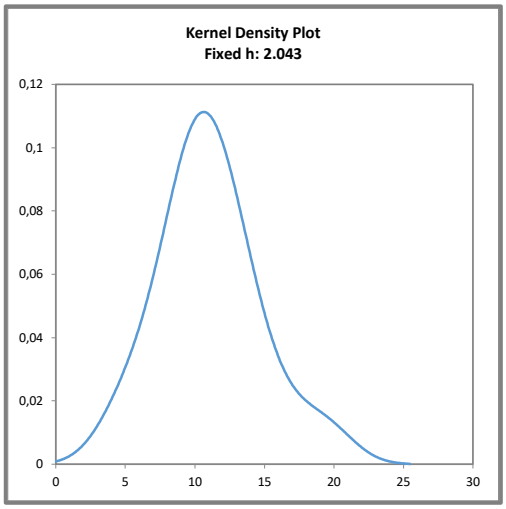


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a slight shoulder approx. 20 mg/kg (method ET).

Characteristics: Quantitative evaluation ELISA Cashew

Sample A

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	17	9
Number of outliers	0	0
Mean	11,1	10,2
Median	11,0	10,1
Robust Mean (X)	**10,9**	**10,1**
Robust standard deviation (S*)	**3,27**	**2,95**
Target range:		
Target standard deviation σ_{pt}	**2,72**	**2,53**
Target standard deviation (for Information)	2,90	2,81
lower limit of target range	**5,45**	**5,06**
upper limit of target range	**16,3**	**15,2**
Quotient S/σ_{pt}*	*1,2*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*0,993*	*1,23*
Results in the target range	*15*	*7*
Percent in the target range	*88*	*78*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation shows nearly a symmetrical distribution of results.

The evaluation of all methods and of method RS-F showed a normal variability of results. 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 100% of the spiking level of almond to sample A and within the range of the recommendations for the applied methods (s. 3.4.3 and p.43 "Recovery rates ELISA for Cashew").

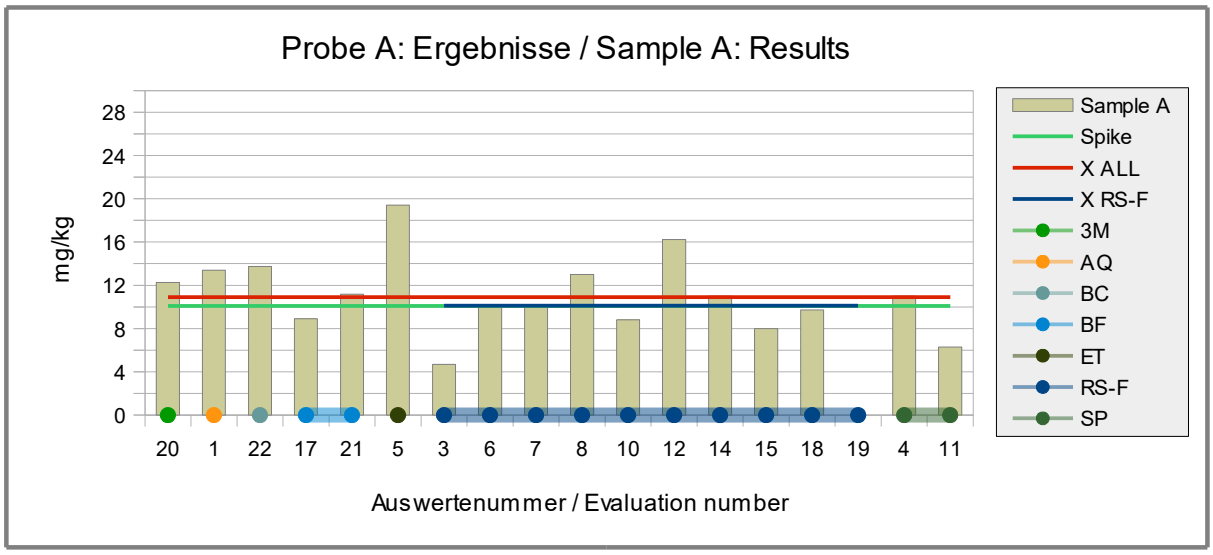


Abb./Fig. 12: ELISA Results Cashew
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

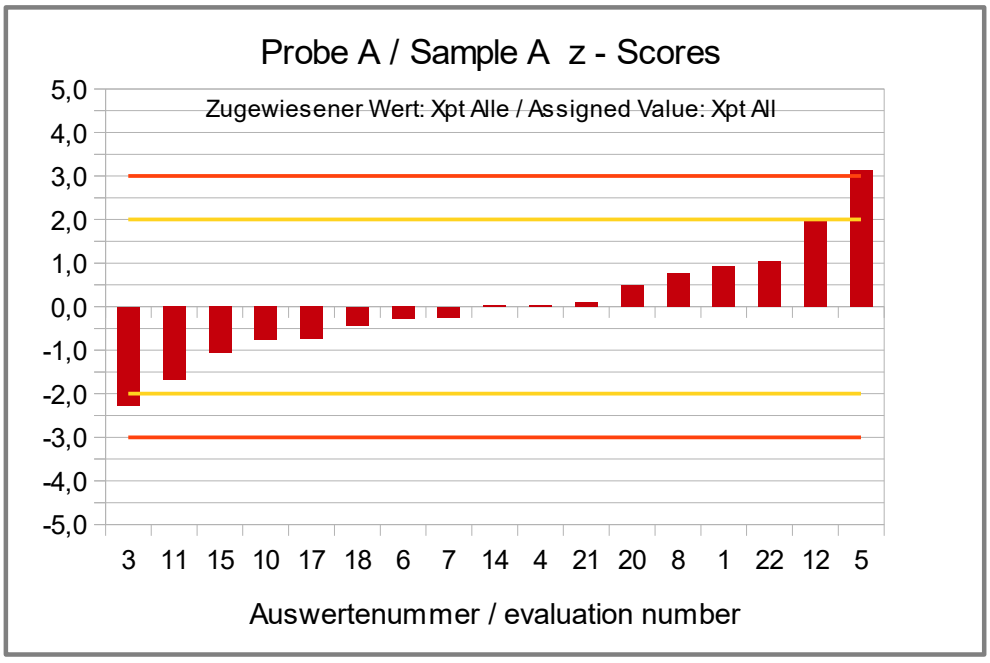


Abb./Fig. 13:
z-Scores (ELISA Results Cashew)
Assigned value robust mean of all results

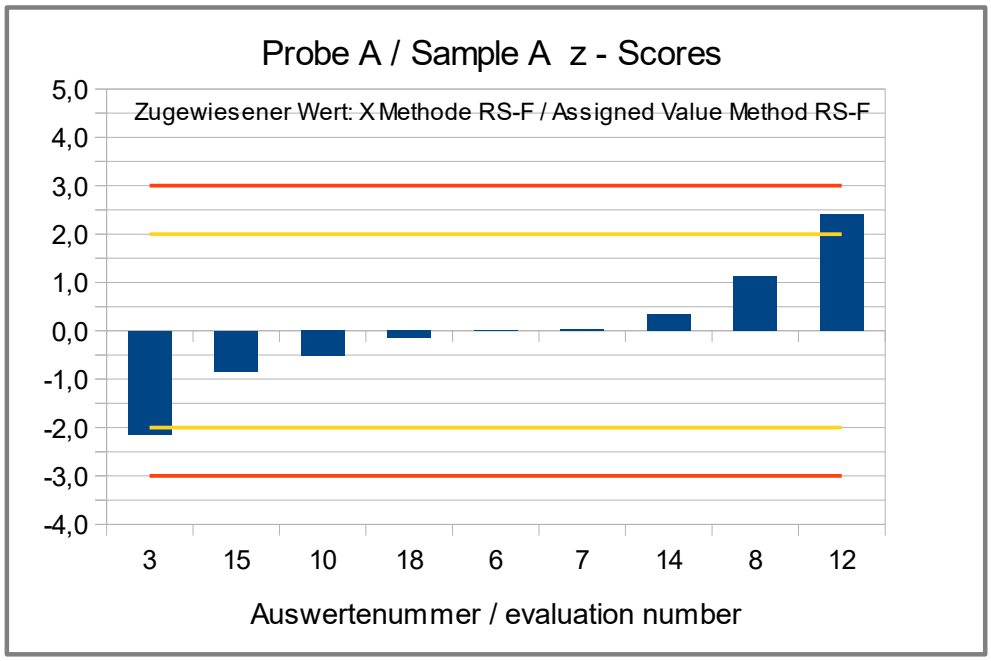


Abb./Fig. 14:
z-Scores (ELISA Results Cashew)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Spiking Level Sample	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
20	24,8	2,1		3M	
1	26,0	2,4		AQ	
22	20,9	1,1		BC	
17	14,3	-0,5		BF	
21	10,2	-1,5		BF	
5	21,5	1,3		ET	Results converted °
3	7,70	-2,1	-1,95	RS-F	
6	15,2	-0,29	0,03	RS-F	
7	15,5	-0,20	0,13	RS-F	
8	19,0	0,65	1,05	RS-F	
10	15,5	-0,21	0,12	RS-F	
12	19,0	0,65	1,1	RS-F	
14	16,0	-0,09	0,26	RS-F	
15	12,0	-1,1	-0,81	RS-F	
18	13,2	-0,8	-0,50	RS-F	
4	13,0	-0,8		SP	
11	16,0	-0,1		SP	

° Calculation see p. 19

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ET = Elution Technologies ELISA Kit
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

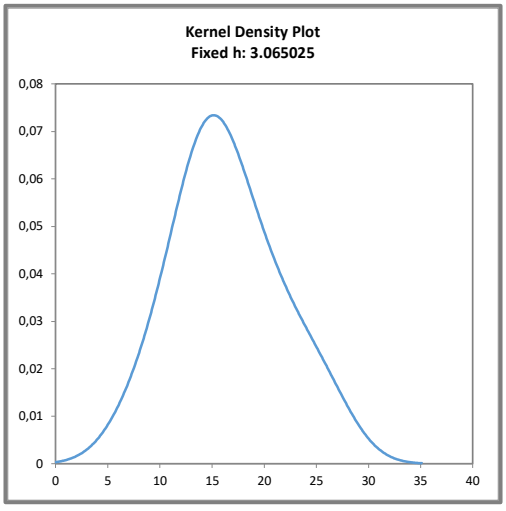


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results.

Characteristics: Quantitative evaluation ELISA Cashew

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	17	9
Number of outliers	0	0
Mean	16,5	14,8
Median	15,5	15,5
Robust Mean (X)	**16,3**	**15,0**
Robust standard deviation (S*)	**5,07**	**3,39**
Target range:		
Target standard deviation σ_{pt}	**4,09**	**3,76**
lower limit of target range	**8,17**	**7,52**
upper limit of target range	**24,5**	**23**
Quotient S/σ_{pt}*	*1,2*	*0,90*
Standard uncertainty $U_{(Xpt)}$	*1,54*	*1,41*
Results in the target range	*14*	*9*
Percent in the target range	*82*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation shows nearly a symmetrical distribution of results.

The evaluation of all methods and of method RS-F showed a normal to low variability of results. 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 133% and 122% 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.43 "Recovery rates ELISA for Cashew").

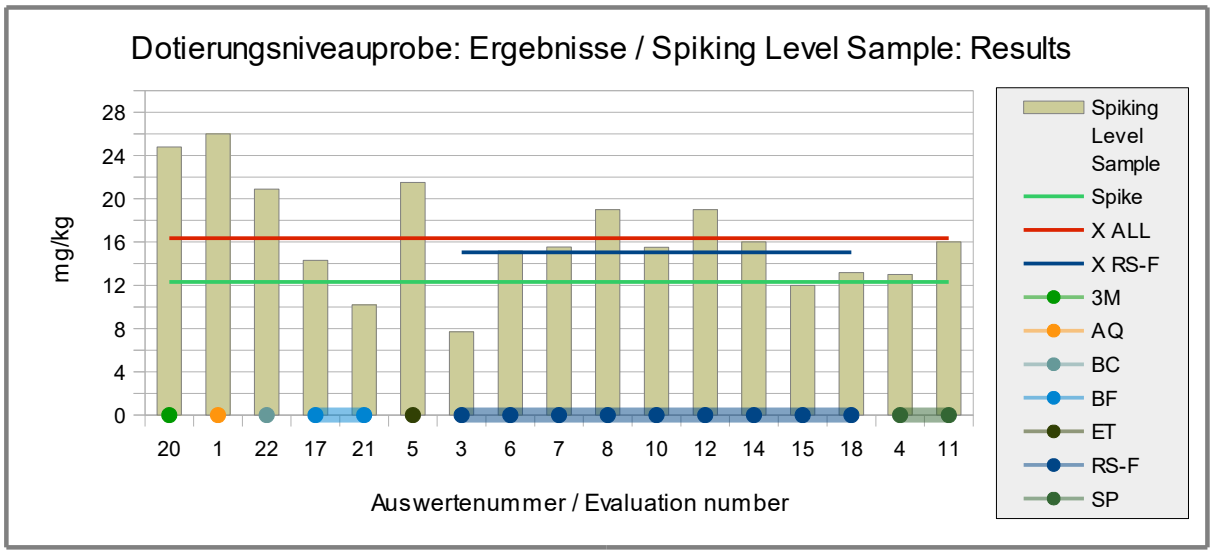


Abb./Fig. 16: ELISA Results Cashew
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

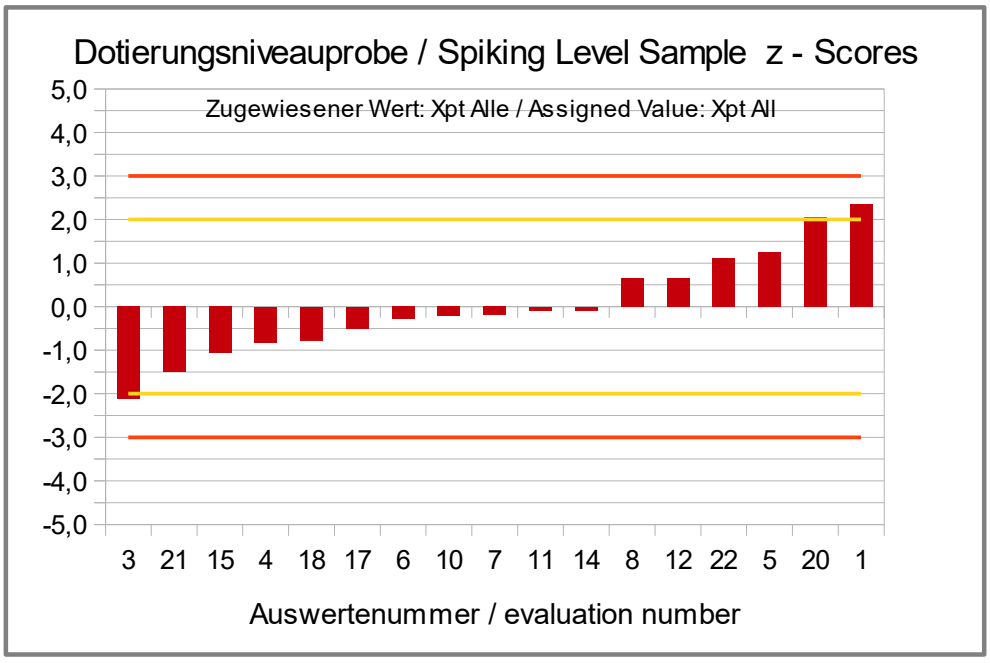


Abb./Fig. 17:
z-Scores (ELISA Results Cashew)
Assigned value robust mean of all results

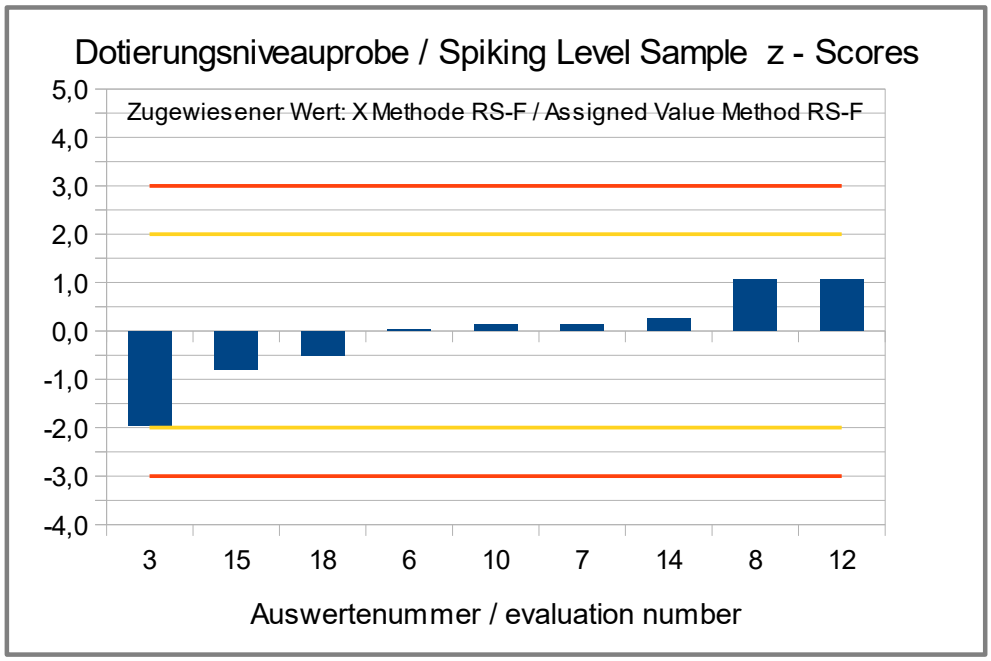


Abb./Fig. 18:
z-Scores (ELISA Results Cashew)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates with z-Scores ELISA for Cashew: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
20	24,8	201	4,1	12,3	**121**	0,85	3M	
1	26,0	211	4,5	13,4	**133**	1,3	AQ	
22	20,9	170	2,8	13,7	**136**	1,4	BC	
17	14,3	**116**	0,65	8,90	**88**	-0,48	BF	
21	10,2	**83**	-0,69	11,2	**111**	0,43	BF	
5	21,5	175	3,0	19,4	192	3,7	ET	Results converted °
3	7,70	**63**	-1,5	4,70	47	-2,1	RS-F	
6	15,2	**123**	0,93	10,1	**100**	0,01	RS-F	
7	15,5	**126**	1,1	10,2	**101**	0,05	RS-F	
8	19,0	154	2,2	13,0	**129**	1,1	RS-F	
10	15,5	**126**	1,0	8,80	**87**	-0,51	RS-F	
12	19,0	154	2,2	16,2	161	2,4	RS-F	
14	16,0	**130**	1,2	11,0	**109**	0,36	RS-F	
15	12,0	**98**	-0,10	8,00	**79**	-0,83	RS-F	
18	13,2	**107**	0,28	9,73	**96**	-0,15	RS-F	
4	13,0	**106**	0,23	11,0	**109**	0,36	SP	
11	16,0	**130**	1,2	6,30	**62**	-1,5	SP	

° Calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**14**
Percent in RA	**65**	Percent in RA	**82**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ET = Elution Technologies ELISA Kit
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comments:

65% (11) 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 spiked food matrix sample A 82% (14) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.2.2 PCR Results: Cashew

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		
9	positive		negative		2/2 (100%)	SFA	
22	positive	25,3	negative	<1	2/2 (100%)	SFA	
16	positive		negative		2/2 (100%)	SFA-Q	
3	negative		negative		1/2 (50%)	div	
7	positive		negative		2/2 (100%)	div	
13a	positive	2,60	negative		2/2 (100%)	div	
13b	positive	2,70	negative		2/2 (100%)	div	
13c	positive	2,40	negative		2/2 (100%)	div	
19	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	8		0	
Number negative	1		9	
Percent positive	89		0	
Percent negative	11		100	
Consensus value	positive		negative	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode

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

Quantitative Valuation PCR: Sample A

An evaluation of the quantitative results was not carried out because too few results were available

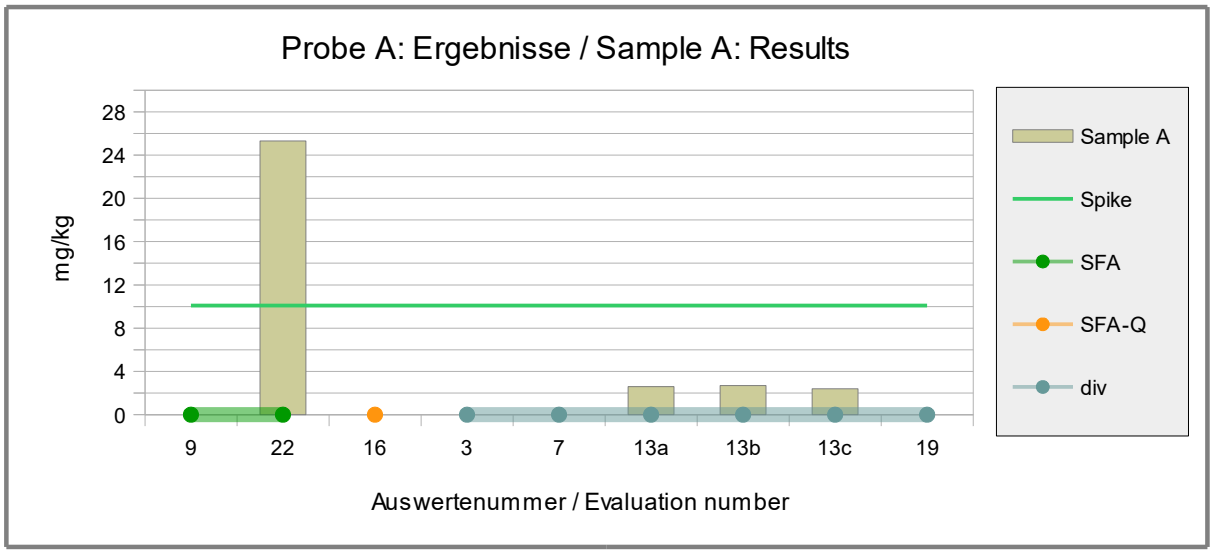


Abb./Fig. 19: PCR Results Cashew
green line = Spiking level
round symbols = Applied methods (see legend)

Qualitative valuation PCR: Spiking Level Sample

An evaluation of the quantitative results was not carried out because too few results were available

Evaluation number	Cashew	Cashew	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
22	positive	23,8		SFA	
13a	positive	2,90		div	
13b	positive	3,20		div	
13c	positive	3,10		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

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

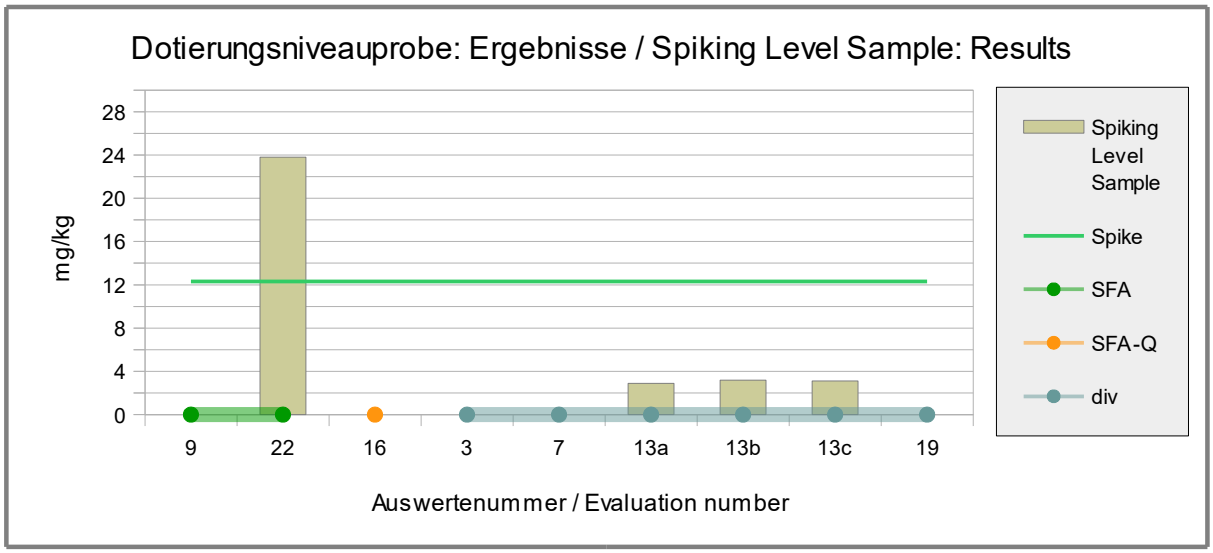


Abb./Fig. 20: PCR Results Cashew
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Cashew: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
22	23,8	193	3,7	25,3	250	6,0	SFA	
13a	2,90	24	-3,1	2,60	26	-3,0	div	
13b	3,20	26	-3,0	2,70	27	-2,9	div	
13c	3,10	25	-3,0	2,40	24	-3,0	div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

Comments:

Four quantitative PCR results were submitted for cashew. The recovery rates for the spiking level sample as well as for the spiked food matrix sample A were either well below (3 results) or weill above (1 result) the AOAC requirement of 50-150%.

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

4.3 Participant z-Scores: overview table

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

Evaluation number	ELISA Mandel: **Xpt** (div. Methods)		ELISA Mandel: **Xpt** (Method: RS-F)		ELISA Cashew: **Xpt** (div. Methods)		ELISA Cashew: **Xpt** (Method: RS-F)	
	Sample A*	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1		0,95	-0,26	0,45	0,92	2,40		
2		8,3						
3		0,95	0,09	0,45	-2,3	-2,1	-2,1	-2,0
4	1,1	-0,70			0,04	-0,82		
5	-0,12	-0,19			3,1	1,3		
6		0,14	-0,19	-0,27	-0,29	-0,29	0,00	0,03
7	-0,13	-1,0			-0,25	-0,20	0,04	0,13
8	0,66	-1,2			0,77	0,65	1,1	1,1
9								
10		0,38	-0,10	-0,06	-0,77	-0,21	-0,52	0,12
11	-0,06	-1,2			-1,7	-0,09		
12					2,0	0,65	2,4	1,1
13	-0,5	-0,94						
14		0,01	0,13	-0,39	0,04	-0,09	0,35	0,26
15		-0,70	-1,0	-1,0	-1,1	-1,1	-0,84	-0,81
16		1,1	0,09	0,58				
17	-0,53	0,12			-0,73	-0,50		
18			0,36		-0,43	-0,78	-0,15	-0,50
19								
20	-0,19	-0,44			0,50	2,1		
21		-0,19	-0,12	-0,57	0,10	-1,5		
22		1,4	1,4	0,83	1,0	1,1		

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

* Methods of „Peak10“ (see chapter 4.1.1, page 21ff).

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 Almond: Xpt (Spiked Level)		ELISA Cashew: Xpt (Spiked Level)		PCR Almond: Xpt (Spiked Level)		PCR Cashew: Xpt (Spiked Level)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1	-0,61	0,54	1,3	4,5				
2	3,3	7,3						
3	-0,29	0,54	-2,1	-1,5				
4	-1,5	-1,0	0,36	0,23				
5	-2,1	-0,50	3,7	3,0				
6	-0,54	-0,19	0,01	0,93				
7	-2,1	-1,2	0,05	1,1				
8	-1,7	-1,4	1,1	2,2				
9								
10	-0,46	0,02	-0,51	1,0				
11	-2,1	-1,4	-1,5	1,2				
12			2,4	2,2				
13/13a	-2,3	-1,2					-3,0	-3,1
13b					-3,9	-3,9	-2,9	-3,0
13c					-3,6	-3,5	-3,0	-3,0
14	-0,25	-0,32	0,36	1,2				
15	-1,3	-1,0	-0,83	-0,10				
16	-0,29	0,67						
17	-2,3	-0,21	-0,48	0,65				
18			-0,15	0,28				
19			0,00	0,00				
20	-2,1	-0,73	0,85	4,1				
21	-0,49	-0,50	0,43	-0,69				
22	0,86	0,93	1,4	2,8			6,0	3,7

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: 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
AQ	7	05.01.21	positive	9,145	negative		positive	12,73	0,4			Almond	AgraQuant ELISA Almond COKAL0748, RomerLabs
IL	2	11.12.20	positive	7,4	negative	0,2	positive	11	0,2	0,4	6,24	Almond protein	Immunolab Almond ELISA
NL	8	08.12.20	positive	11	negative	<1	positive	12	1	5	34	Almond	nutriLinia® Almond-E ELISA
NL	13	26.01.21	positive	8,3	negative	<0,2	positive	13	0,2	0,5	30	Almond	nutriLinia® Almond-E ELISA
RS-F	1	10.12.	positive	16,3	negative	<0,1	positive	21	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	3	08.12.20	-	17,8	-	<2.5	-	21	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	6	13.01.21	positive	16,6	negative	<2,5	positive	17,59		2,5	40	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	10	08.01.21	positive	17	negative	<2,5	positive	18,6		2,5	37	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	14	19.01.21	positive	18	negative	<2.5	positive	17	0,3	1		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	15	01.02.21	positive	13	negative	<2.50	positive	14	0,3	1		Please select!	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	16	10.02.21	positive	17,8	negative		positive	21,6	2,5	2,5	31,3	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	18		positive	19,005	negative	<2,5	positive	>20,00	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	19		positive		negative		positive		1,2	2,5		Please select!	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	21	11.01.21	-	16,88	-	<2.50	-	16,18	0,1	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	22	11.02.20	positive	23,32	negative	<2.5	positive	22,79	2,5	2,5	25,99	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
SP	4	18.12.20	positive	12	negative	0	positive	14	0,2	0,4		Almond	Eurofins SensiSpec Almond ELISA Kit
SP	11	28.12.20	positive	9,3	negative	<0,4	positive	12	0,4	0,4		Almond	Eurofins SensiSpec Almond ELISA Kit
VT	5	08.01.21	positive	9,16	negative	<2,5	positive	16,19		2,5		Almond	Veratox Almond, Neogen
VT	17	02.10.21	Detected	8,2	Not detected	<2.5	Detected	17,5		2,5		Food	Veratox, Neogen
VT	20	09.02.21	positive	9.0	negative		positive	15,1		2,5		Almond	Veratox Almond, Neogen

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

Continuation ELISA Almond:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	7			yes	
IL	2	Almond protein	Extraction: 1 g of homogenized mixture suspended in 20 mL of pre-diluted extraction and sample dilution buffer/ 15 minutes of sample incubation in 60°C/10 minutes of 2000 x g centrifugation Determination: 100 µL of particle-free solution, ready-to-use standards applied per well/ 20 minutes incubation at room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL conjugate into each well/ 20 minutes incubation in room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL substrate solution into each well/ 20 minutes incubation in the dark, at room temperature/ add 100 µL Stop enzyme solution into each well/ Measure absorbance at 450 nm (reference 620 nm)	No	
NL	8		as per kit instructions	yes	
NL	13		as per kit instructions	yes	
RS-F	1	Almond proteins	Allergen extraction buffer (Kit), 10 min, 60°C	yes	
RS-F	3			yes	
RS-F	6				
RS-F	10			Yes	
RS-F	14		as per kit instructions	yes	
RS-F	15		as per kit instructions	yes	
RS-F	16	see manual	as per kit instructions	yes	
RS-F	18				
RS-F	19			YES	
RS-F	21			No	
RS-F	22	As per kit instructions	As per kit instructions	Yes	
SP	4				
SP	11	detects almond proteins	as per kit instructions	yes	
VT	5		as per kit insert	yes	
VT	17			yes	
VT	20		PBS / 15 mins/ 60 degrees	yes	

5.1.2 ELISA: Cashew

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
3M	20	04.02.21	positive	12,25	negative		positive	24,77		0,9		total food item	3M (Method no. E96CHW)
AQ	1	10.12.	positive	13,4	negative	<0,2	positive	26	0,2	2		Cashew	RomerLabs , AgraQuant 10002094
BC	22	11.12.20	positive	13,74	negative	<2	positive	20,89	2	2	30,43	Cashew	BioCheck ELISA Cashew-Check
BF	17	26/01	Detected	8,9	Not detected	<2.0	Detected	14,3		2		Food	Monotrace, BioFront
BF	21	17/12	-	11,18	-	<2.00	-	10,19	0,12	2		Cashew	MonoTrace Cashew ELISA kit, BioFront Technologies
ET	5	06.01.21	positive	3,59	negative	<0,90	positive	3,98		0,9		Cashew protein	Elution Technologies ELISA Kit Cashew Protein E-75CSH
RS-F	3	17.12.20	-	4,7	-	<2.5	-	7,7	0,2	2,5		Cashew	andere: bitte eingeben!
RS-F	6	12.01.21	positive	10,12	negative	<2,5	positive	15,16		2,5	40	Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	7	22.01.21	positive	10,215	negative		positive	15,536	5			Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	8	14.12.20	positive	13	negative	<1	positive	19	1	5	22	Cashew	Ridascreen® FAST Cashew R6872, R-Biopharm
RS-F	10	12.01.21	positive	8,8	negative	<2,5	positive	15,5		2,5		Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	12		positive	16,22	negative	-	positive	19	0,2	2,5		Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	14	19.01.21	positive	11	negative	<2.5	positive	16	0,13	2,5		Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	15	01.02.21	positive	8	negative	<2.50	positive	12	0,1	2,5		Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	18		positive	9,73	negative	<2,5	positive	13,17	0,13	2,5		Cashew	Ridascreen® FAST Cashew R7862, R-Biopharm
RS-F	19		positive		negative		positive		1,2	2,5		Please select!	Ridascreen® FAST Cashew R7862, R-Biopharm
SP	4	18.12.20	positive	11	negative	0	positive	13	0,2	2		Cashew	Eurofins SensiSpec Cashew ELISA Kit
SP	11	13.01.21	positive	6,3	negative	<2	positive	16	1,5	2		Cashew	Eurofins SensiSpec Cashew ELISA Kit

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

Continuation ELISA Cashew:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
3M	20		3M Extraction buffer/25 mins/ 60 degrees	yes	19.1% protein content
AQ	1	Cashew proteins	Allergen extraction buffer (Kit), 15 min, 60°C	yes	
BC	22	As per kit instructions	As per kit instructions	Yes	
BF	17			yes	
BF	21			No	
ET	5		as per kit insert	yes	
RS-F	3		Ridascreen FAST Cashew R 6872 (w rong number in pull dow n list?)	yes	Ridascreen FAST Cashew R 6872
RS-F	6				
RS-F	7			yes	
RS-F	8		as per kit instructions, w ithout milk protein	yes	
RS-F	10			Yes	Correct method: Ridascreen FAST Cashew R6872, Rbiopharm
RS-F	12			Yes	Lot used: 23300
RS-F	14		as per kit instructions	yes	
RS-F	15		as per kit instructions	yes	
RS-F	18				
RS-F	19			YES	
SP	4				
SP	11	detects Cashew proteins	as per kit instructions	yes	

5.1.3 PCR: Almond

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit+Manufacturer
SFA	7	14.12.20	positive		negative		positive					Almond-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9		positive		negative		positive		0,4			Almond	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16	18.01.21	positive		negative		positive		0,4			Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	3	16.12.20	-	neg.	-	neg.	-	pos.				Almond-DNA	
div	13a	27.01.21	positive	<5 (2)	negative		positive	<5 (1,4)	5		40	Almond	Real Time PCR
div	13b	27.01.21	positive	0,36	negative		positive	0,47	0,05		40	Almond	Real Time PCR
div	13c	27.01.21	positive	2	negative		positive	2,2	0,05		40	Almond	Real Time PCR
div	19		negative		negative		positive		0,008	0,08		Please select!	internal method

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
SFA	7		Macherey & Nagel NucleoSpin Food Kit, 2 g sample w eight	yes	
SFA	9			no	
SFA	16	see test kit	SureFood® PREP Advanced, Congen / R-Biopharm	yes	
div	3	PRU AV1	internal method. 3-plex RT-qPCR	yes	Screening method
div	13a	nsLTP	Standard 1 (Quantard 40)	yes	semi quantitative (1-point calibration)
div	13b	Chloroplast	Standard 1 (Quantard 40)	yes	semi quantitative (1-point calibration)
div	13c	Chloroplast	Standard 2 (rice cookie)	yes	semi quantitative (1-point calibration)
div	19			YES	

5.1.4 PCR: Cashew

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
SFA	9		positive		negative		positive		0,4			Cashew	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	22	04.01.21	positive	25,3	negative	<1	positive	23,8	1	1	40	Cashew	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q	16	05.01.21	positive		negative		positive		0,4			Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	3	16.12.20	-	neg.	-	neg.	-	pos.				Cashew-DNA	andere: bitte eingeben!
div	7	14.12.20	positive		negative		positive					Cashew-DNA	Köppel et al, „Two quantitative hexaplex real-time PCR systems for the detec-tion and quantification of DNA from twelve allergens in food“, Eur Food Res Technol, 2012
div	13a	27.01.21	positive	2,6	negative		positive	2,9	2,5		40	Cashew	Real Time PCR
div	13b	27.01.21	positive	2,7	negative		positive	3,2	0,05		40	Cashew	Real Time PCR
div	13c	27.01.21	positive	2,4	negative		positive	3,1	0,05		40	Cashew	Real Time PCR
div	19		positive		negative		positive		0,008	0,08		Please select!	internal method

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
SFA	9			no	
SFA	22	As per kit instructions	As per kit instructions	No	
SFA-Q	16	see test kit	SureFood® PREP Advanced, Congen / R-Biopharm	yes	
div	3	Ana o3	internal method. 3-plex RT-qPCR	yes	Screening method
div	7		Macherey & Nagel NucleoSpin Food Kit, 2 g sample w eight	yes	
div	13a	Ana o3 of the 2S albumin family	Standard 1 (Quantard 40)	yes	semi quantitative (1-point calibration)
div	13b	ITS2	Standard 1 (Quantard 40)	yes	semi quantitative (1-point calibration)
div	13c	ITS2	Standard 2 (rice cookie)	yes	semi quantitative (1-point calibration)
div	19			YES	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL08 Sample A

Weight whole sample	2,80	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	17,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	33	13,2
2	5,00	35	14,0
3	5,01	38	15,2
4	5,03	49	19,5
5	5,03	41	16,3
6	5,01	45	18,0
7	5,01	37	14,8
8	4,98	38	15,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	39,5	Particles
Standard deviation	5,22	Particles
χ^2 (CHI-Quadrat)	4,83	
Probability	**68**	%
Recovery rate	88	%

Normal distribution		
Number of samples	8	
Mean	15,8	mg/kg
Standard deviation	2,08	mg/kg
rel. Standard deviaton	13,2	%
Horwitz standard deviation	10,6	%
HorRat-value	**1,3**	
Recovery rate	88	%

Microtracer Homogeneity Test
DLA ptAL08 Spiking Level Sample

Weight whole sample	1,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	13,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	35	14,0
2	4,97	34	13,7
3	5,02	37	14,7
4	4,99	37	14,8
5	5,00	38	15,2
6	4,98	34	13,7
7	5,01	35	14,0
8	5,03	34	13,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	35,5	Particles
Standard deviation	1,58	Particles
χ^2 (CHI-Quadrat)	0,49	
Probability	**100**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	14,2	mg/kg
Standard deviation	0,63	mg/kg
rel. Standard deviaton	4,5	%
Horwitz standard deviation	10,7	%
HorRat-value	**0,42**	
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	***ptAL08 - 2020***
PT name	***Allergens VIII: Almond and Cashew in Veggie Burger Powder***
Sample matrix (processing)	***Samples A + B:*** *Veggie Burger Powder / ingredients:. Whole oat flakes, wholegrain spelled semolina, wholegrain spelled flakes, corn flakes (corn flour, raw cane sugar, sea salt), onions, leeks, carrots, sunflower oil, sea salt, tomato powder, zucchini, garlic, spices (pepper, mace, turmeric), herbs (lovage leaves, oregano), 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:* *Almond (Almond protein, DNA), Cashew (Cashew 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 February 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.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SPANIEN/SPAIN
		SPANIEN/SPAIN
		KANADA/CANADA
		KANADA/CANADA
		Deutschland/Germany
		Deutschland/Germany
		FRANKREICH/FRANCE
		POLEN/POLAND
		Deutschland/Germany
		Deutschland/Germany
		SCHWEIZ/SWITZERLAND
		SCHWEIZ/SWITZERLAND
		SCHWEIZ/SWITZERLAND
		Deutschland/Germany
		Deutschland/Germany
		KANADA/CANADA
		GROSSBRITANNIEN/GREAT BRITAIN
		Deutschland/Germany
		FRANKREICH/FRANCE
		USA
		VIETNAM
		Deutschland/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.]

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Hor-witz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Ana-lyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estim-ates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messun-gen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Check-ing procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Re-quirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for

Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)

24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 00.00-169 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Erdnuss in Lebensmitteln mittels real-time PCR (2019) [Foodstuffs, detection and determination of peanut in foods by real-time PCR]
33. ASU §64 LFGB L 18.00-20 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of almond (Prunus dulcis) in rice and wheat cookies and sauce powders by PCR]
34. ASU §64 LFGB L 18.00-21 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Paranuss (Bertholletia exceisa) in Reis- und Weizenkeksen sowe in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of brazil nut (Bertholletia exceisa) in rice and wheat cookies and sauce powders by PCR]
35. ASU §64 LFGB L 18.00-22 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]