Evaluation Report

proficiency test

DLA ptAL07 (2020)

Allergens VII:

Crustaceae, Peanut and Coconut

in Instant Product (Asian Noodle Soup)

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1st Correction 28/04/2021:

Regarding the evaluation of the ELISA results for Crustaceae, the results of participants 13 and 14 were not or incorrectly converted from DLA to Crustaceae protein. These errors have been corrected on pages 21-28.
The corrections do not affect the evaluations of other participant results.

On page 25, the incorrect results (from sample B) were reported for the spiking level sample. This has been corrected.

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (28 April 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: 28 April 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 instant noodle soup. The basic composition of samples A and 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:
The spiking materials containing the allergenic ingredients King Prawns, peanuts and coconut flour were crushed, sieved and added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 4 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 homogenised.

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**
Asian instant noodle soup Ingredients: Noodles 89% (wheat flour, potato starch, palm oil, salt, acidity regulators: E501, E500, E339, antioxidant E306, emulsifier E322, seasoning), spice powder 9% (hydrolyzed vegetable protein, maltodextrin, yeast extract, salt, wheat flour, black pepper, garlic, corn flour), vegetable-mushroom flakes 2% (pak choi, shitake, textured vegetable protein, carrots, red chili peppers, onions). Nutrients per 100 g: Fat 13 g, Carbohydrates 68 g, Protein 8,2 g, Salt 3,8 g	100 g/100 g	99,9 g/100g	
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***	–	 12,9 mg/kg 2,99 mg/kg	 15,1 mg/kg 3,50 mg/kg
Coconut flour: – as Coconut flour* – *thereof 17% total protein***	–	 82,9 mg/kg 14,1 mg/kg	 81,2 mg/kg 13,8 mg/kg
King Prawns, freeze-dried – as King Prawns, fresh* – as King Prawns, freeze-dried* – *thereof 87,0% total protein***	–	 242 mg/kg 52,3 mg/kg 45,4 mg/kg	 231 mg/kg 50,9 mg/kg 43,4 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; the corresponding fresh wheight of King Prawns was calculated with a dry matter of 21,6% (USDA Crustaceae)
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=6,25 for Coconut and King Prawns, and F=5,46 for peanuts)

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 88% and 92%, respectively. Additionally, particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat value of 0,93 and 0,89, respectively. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample B by all ELISA tests for Crustaceae (Immunolab, Morinaga and AgraQuant) and Peanut (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].

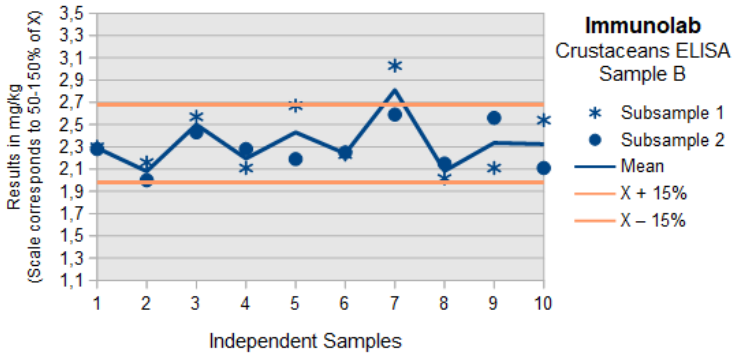
ELISA-Tests: Homogenität Crustaceae / Homogeneity Crustaceae

Immunolab Crustaceans ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomyosin 2,33 ± 0,19 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	2,30	2,28	2,29
2	2,16	2,00	2,08
3	2,57	2,43	2,50
4	2,11	2,28	2,20
5	2,67	2,19	2,43
6	2,23	2,25	2,24
7	3,03	2,59	2,81
8	2,02	2,15	2,08
9	2,11	2,56	2,34
10	2,54	2,11	2,33

General average X	2,33	
SD of sample means Sx	0,22	9,3%
SD within-samples Sw	0,15	6,5%
SD between-samples Ss	0,19	8,1%

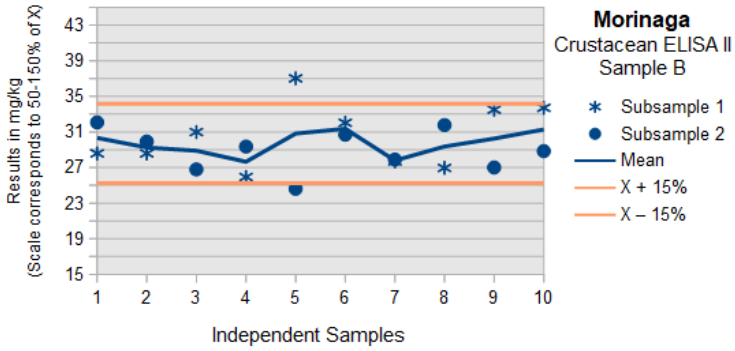


Morinaga Crustacean ELISA II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Crustacean protein 29,7 ± 1,33 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	28,6	32,1	30,3
2	28,6	29,9	29,3
3	31,0	26,8	28,9
4	25,9	29,4	27,7
5	37,0	24,6	30,8
6	32,0	30,7	31,4
7	27,7	27,9	27,8
8	27,0	31,8	29,4
9	33,5	27,0	30,3
10	33,7	28,9	31,3

General average X	29,7	
SD of sample means Sx	1,33	4,5%
SD within-samples Sw	2,68	9,0%
SD between-samples Ss	<1,33	<4,5%

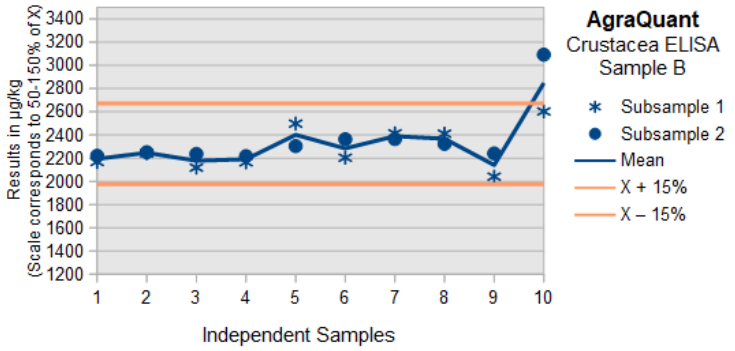


AgraQuant Crustacea ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomyosin 2320 ± 195 µg/kg

Sample B	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	2168	2220	2194
2	2242	2251	2247
3	2119	2237	2178
4	2165	2217	2191
5	2498	2304	2401
6	2204	2363	2284
7	2414	2364	2389
8	2411	2323	2367
9	2041	2241	2141
10	2602	3092	2847

General average X	2324	
SD of sample means Sx	206	8,9%
SD within-samples Sw	96	4,1%
SD between-samples Ss	195	8,4%



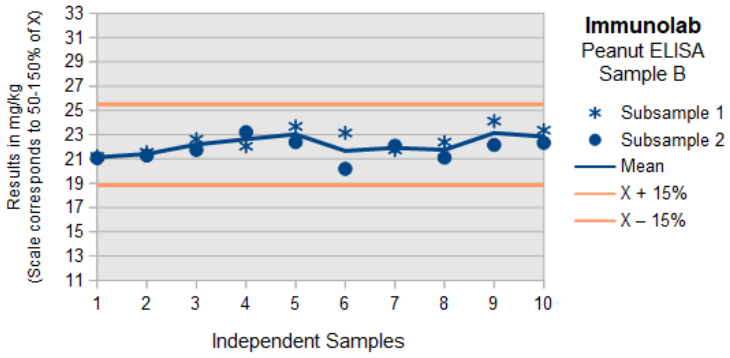
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 22,2 ± 0,51 mg/kg

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

General average X	22,2	
SD of sample means Sx	0,706	3,2%
SD within-samples Sw	0,694	3,1%
SD between-samples Ss	0,508	2,3%

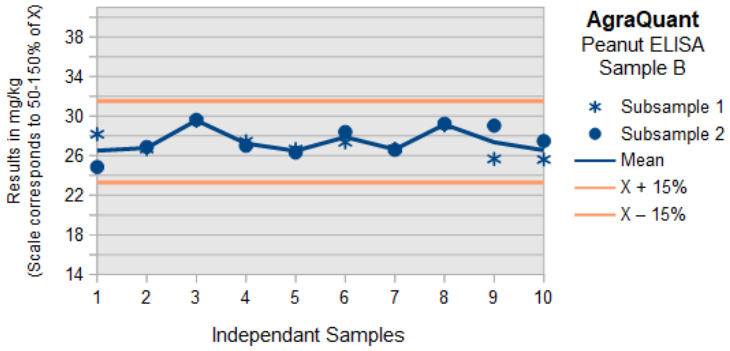


AgraQuant Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 27,4 ± 0,96 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	28,2	24,9	26,5
2	26,7	26,9	26,8
3	29,5	29,6	29,6
4	27,5	27,0	27,2
5	26,7	26,3	26,5
6	27,4	28,4	27,9
7	26,7	26,6	26,6
8	29,1	29,2	29,1
9	25,7	29,0	27,4
10	25,6	27,5	26,5

General average X	27,4	
SD of sample means Sx	1,12	4,1%
SD within-samples Sw	0,826	3,0%
SD between-samples Ss	0,959	3,5%



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 values of sample B and the spiking level sample were approx. 0,40 and 0,41 (18°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 45th week of 2020. The testing method was optional. The tests should be finished at 8th January 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 Crustaceae, Peanut and Coconut in the range of mg/kg in the matrix of Asian Soup (Instant Product). 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 limit of quantification, specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 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 material 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 evaluated 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]**	**Recov-ery**	**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** were converted by DLA to **total food items (peanut)** using the analyzed protein content of the raw materials (see page 5).

The **ELISA results** for Crustaceae were evaluated as Crustaceae protein. Depending on the applied test kit, results given as the fresh product have been converted directly into Crustaceae protein (test kit manual: Ridascreen with 20% protein, handbook: Veratox with 22,78% protein) or first in Crustaceae, dried (AgraQuant, BioFront). In the second case, a dry weight of 21,6% (literature: Crustacean, Shrimps roh, FoodData Central, USDA 2021) was taken as a basis and then converted to the total protein content using the experimentally determined protein content of 87% in the dry matter (see p.5).
Results given as Crustaceae dry weight (Ridascreen) have also been converted to the total protein content with the experimentally determined protein content of 87% in dry matter (see p.5).

ELISA results, expressed as tropomyosin, have been converted to total protein using the manufacturer's data of 20% tropomyosin in total protein (AgraQuant, Immunolab, Eurofins).

Results given as coconut fresh (ELISA Immunolab) or indicated as such according to the manufacturer's instructions (coconut = fresh coconut, personal communication) (Immunolab) have been converted into the dry product coconut flour. This was based on a dry matter of 55,2% (food composition and nutrition tables Souci, Fachmann, Kraut).

The **PCR results** for crustaceae were evaluated as fresh crustaceae. For calculation of the recovery rate the spiked King prawns content was converted into fresh weight by a dry weight of 21,6% (literature: Crustacean, Shrimps roh, FoodData Central, USDA 2021).

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

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

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

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

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

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

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

After that the recovery rates of the results for the spiking level sample

and the spiked sample are reported. The number of results within the range of acceptance of 50-150% is given.

4.1 Proficiency Test Crustaceae

4.1.1 ELISA Results: Crustaceae (as total protein)

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		
10	negative	<0,1	positive	16,3	2/2 (100%)	AQ	
16	negative	<0,2	positive	9,09	2/2 (100%)	AQ	
18	negative	<0.02	positive	1,75	2/2 (100%)	AQ	
20	negative	<LOD	positive	0,624	2/2 (100%)	AQ	Result converted °
19	negative		positive		2/2 (100%)	AS	Result converted °
4	negative	<0,19	positive	>7,5	2/2 (100%)	BF	Result converted °
22	negative	0	positive	139	2/2 (100%)	BF	
12	negative	<0,25	positive	3,98	2/2 (100%)	ES	Result converted °
3	negative		positive	9,85	2/2 (100%)	IL	
7	negative	<20	positive	435	2/2 (100%)	RS-F	Result converted °
8	negative	<17	positive	122	2/2 (100%)	RS-F	
13	negative	<20	positive	77,7	2/2 (100%)	RS-F	Result converted °
14	negative	<4,3	positive	98,0	2/2 (100%)	RS-F	Result converted °
5	negative	<0,1	positive	10,0	2/2 (100%)	SP	Result converted °
15	negative	<0,005	positive	14,5	2/2 (100%)	SP	Result converted °
21	negative	0	positive	13,0	2/2 (100%)	SP	Result converted °
2	negative	<0,57	positive	5,47	2/2 (100%)	VT	Result converted °

° calculation see p. 19

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

Methods:

AQ = AgraQuant, RomerLabs
AS = AgraStrip (Lateral Flow), RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
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

Note: Due to the heterogeneity of quantitative results and the small number of results from single methods no statistical evaluation with z-scores was done.

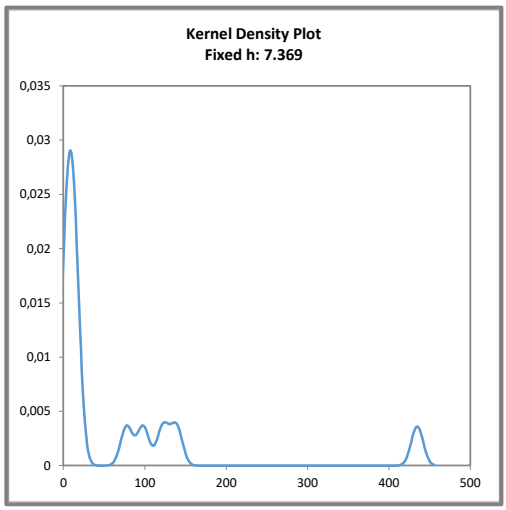


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a maximum < 50 mg/kg for the results obtained from 5 methods (method AQ, ES, IL, SP, VT), the values > 50mg/kg are obtained by 2 methods (BF, RS-F) (see Figure 1).

Characteristics: Quantitative evaluation ELISA: Crustaceae (as total protein)

Note: Due to the heterogeneity of quantitative results and the small number of results from single methods no statistical evaluation with z-scores was done.

Sample B

Statistic Data	**Results <50** *[mg/kg]*	**Results >50** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL<50}}$	$X_{pt_{ALL>50}}$
Number of results	10	5
Number of outliers	–	–
Mean	8,46	174
Median	9,47	122
Robust Mean (X)	8,46	133
Robust standard deviation (S*)	6,08	66,5
Target range:		
Target standard deviation σ_{pt}		
lower limit of target range		
upper limit of target range		
Quotient S/σ_{pt}*		
Standard uncertainty $U_{(Xpt)}$		
Results in the target range		
Percent in the target range		

Comments to the statistical characteristics and assigned values:

The kernel density estimation *showed a maximum < 50 mg/kg for the results obtained from 5 methods (method AQ, ES, IL, SP, VT), the other values > 50mg/kg are obtained by 2 other methods (BF, RS-F).*
A joint evaluation of the results of different methods was not possible. Due to the small number of results, no evaluation was made for the single methods.

The individual quantitative results were evaluated in comparison to the spiking level for information (see "Recovery rates with z-Scores for Crustaceae (as total protein)" p. 28).

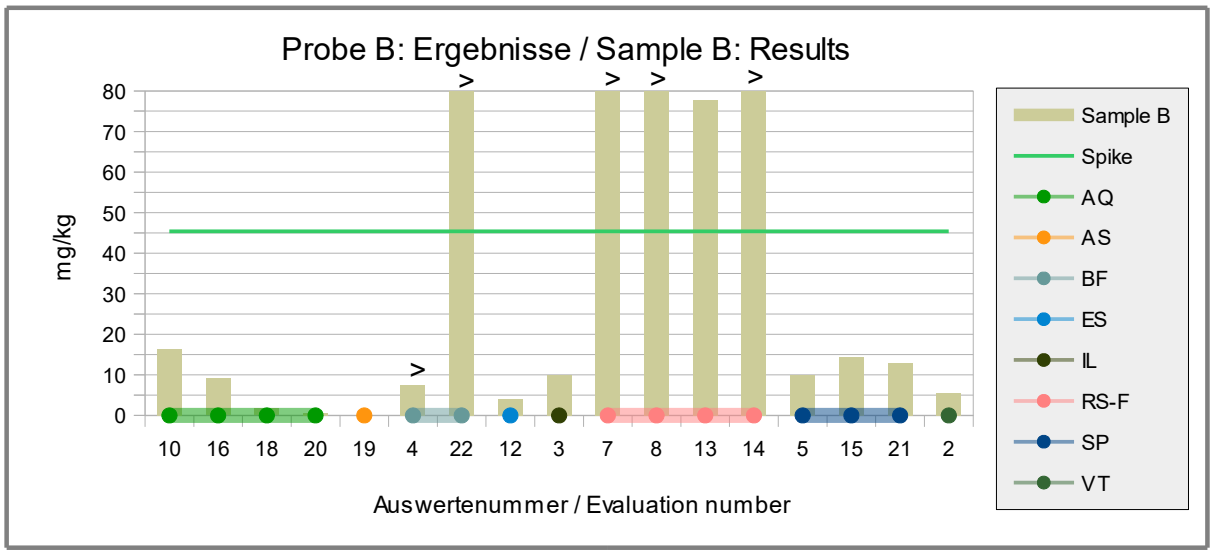


Abb./Fig. 2: ELISA Results Crustaceae (as total protein)
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative valuation of results: Spiking level sample

Note: Due to the heterogeneity of quantitative results and the small number of results from single methods no statistical evaluation with z-scores was done.

Evaluation number	Crustaceae protein	Crustaceae protein	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
10	positive	15,5		AQ	
16	positive	10,2		AQ	
18	positive	1,45		AQ	
20	positive	0,770		AQ	Result converted °
19	positive			AS	Result converted °
4	positive	>7,5		BF	Result converted °
22	positive	41,5		BF	
12	positive	2,65		ES	Result converted °
3	positive	10,4		IL	
7	positive	452		RS-F	Result converted °
8	positive	131		RS-F	
13	positive	90,1		RS-F	Result converted °
14	positive	106		RS-F	Result converted °
5	positive	9,5		SP	Result converted °
15	positive	14,5		SP	Result converted °
21	positive	18		SP	Result converted °
2	positive	5,10		VT	Result converted °

° calculation see p. 19

Number positive	17	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:

AQ = AgraQuant, RomerLabs
AS = AgraStrip (Lateral Flow), RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

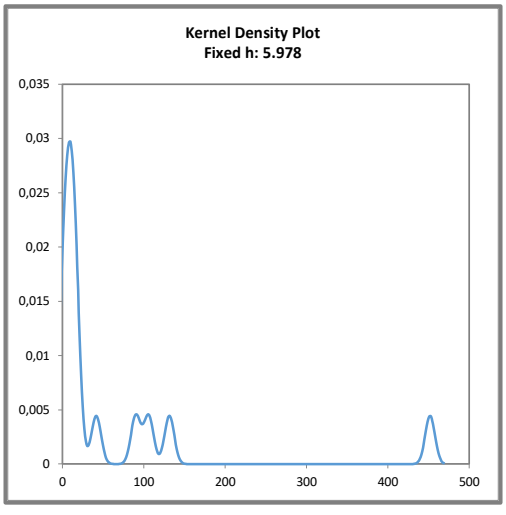


Abb. / Fig. 3:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation showed an inconsistent distribution of the results (see also Fig. 2).

Characteristics: Quantitative evaluation Crustaceae (as total protein)

Note: Due to the heterogeneity of quantitative results and the small number of results from single methods no statistical evaluation with z-scores was done.

Spiking level sample

Statistic Data	**Results <40** *[mg/kg]*	**Results >40** *[mg/kg]*
Assigned value (Xpt)	$\boldsymbol{Xpt_{ALL<40}}$	$\boldsymbol{Xpt_{ALL>40}}$
Number of results	10	5
Number of outliers	-	-
Mean	8,81	164
Median	9,85	106
Robust Mean (X)	8,81	125
Robust standard deviation (S*)	6,94	89,9
Target range:		
Target standard deviation σ_{pt}		
lower limit of target range		
upper limit of target range		
Quotient S/σ_{pt}*		
Standard uncertainty $U_{(Xpt)}$		
Results in the target range		
Percent in the target range		

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a maximum < 40 mg/kg for the results obtained from 5 methods (method AQ, ES, IL, SP, VT), the other values > 40mg/kg are obtained by 2 methods (BF, RS-F).
A joint evaluation of the results of different methods was not possible. Due to the small number of results, no evaluation was made for the single methods.
The individual quantitative results were evaluated in comparison to the spiking level for information (see "Recovery rates with z-Scores for Crustaceae (as total protein)" p. 28).

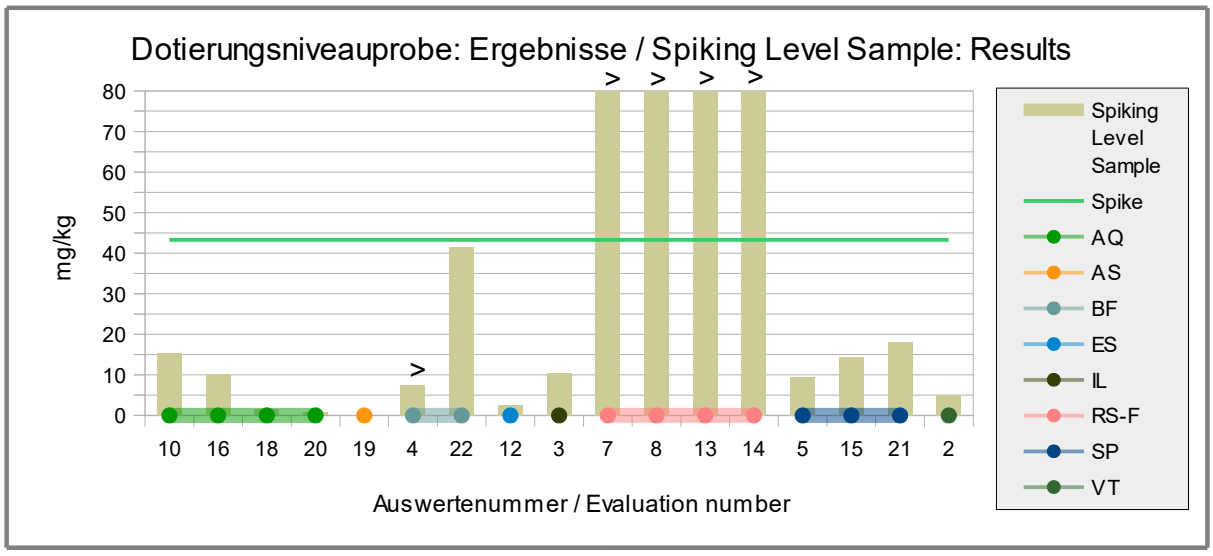


Abb./Fig. 4: ELISA Results Crustaceae (as total protein)
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates ELISA for Crustaceae (as total protein): 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}]		
10	15,5	36	-2,6	16,3	36	-2,6	AQ	
16	10,2	24	-3,1	9,09	20	-3,2	AQ	
18	1,45	3,3	-3,9	1,75	3,9	-3,8	AQ	
20	0,770	1,8	-3,9	0,624	1,4	-3,9	AQ	Result converted °
19							AS	
4	>7,5			>7,5			BF	Result converted °
22	41,5	**96**	-0,17	139	306	8,2	BF	
12	2,65	6	-3,8	3,98	8,8	-3,6	ES	Result converted °
3	10,4	24	-3,0	9,85	22	-3,1	IL	
7	452	1044	38	435	958	34	RS-F	Result converted °
8	131	304	8,1	122	270	6,8	RS-F	
13	90,1	208	4,3	77,7	171	2,8	RS-F	Result converted °
14	106	245	5,8	98,0	216	4,6	RS-F	Result converted °
5	9,5	22	-3,1	10,0	22	-3,1	SP	Result converted °
15	14,5	33	-2,7	14,5	32	-2,7	SP	Result converted °
21	18,0	42	-2,3	13,0	29	-2,9	SP	Result converted °
2	5,10	12	-3,5	5,47	12	-3,5	VT	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	0
Percent in RA	7	Percent in RA	0

* Recovery rate 100% relative size: King prawns protein, s. page 4

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
AS = AgraStrip (Lateral Flow), RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

With one exception, none of the participants has obtained a recovery rate within the range of the AOAC recommendation of 50-150% with the spiking level sample or the spiked food matrix sample B by ELISA methods. The recovery rates can be divided in two groups one below 50% and the other above 150%.

Note: With respect to the recovery rates of ELISA methods for the determination of crustaceans for some methods special conversion factors for specific species have to be considered (e.g. methods: AQ, IL, SP). These factors have not been considered in the present section for determination of king prawns, which are contained in the PT samples.

4.1.2 PCR Results: Crustaceae (fresh)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
4	negative	<0,4	positive		2/2 (100%)	SFA	
9	negative		positive		2/2 (100%)	SFA	
13	negative	<1	positive	356	2/2 (100%)	SFA	
17	negative		positive		2/2 (100%)	SFA	
1	negative		positive	1500	2/2 (100%)	div	
19	negative		positive		2/2 (100%)	div	

° calculation see p. 19

	Sample A		Sample B	
Number positive	0		6	
Number negative	6		0	
Percent positive	0		100	
Percent negative	100		0	
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 evaluation was done, because there were < 5 quantitative results.

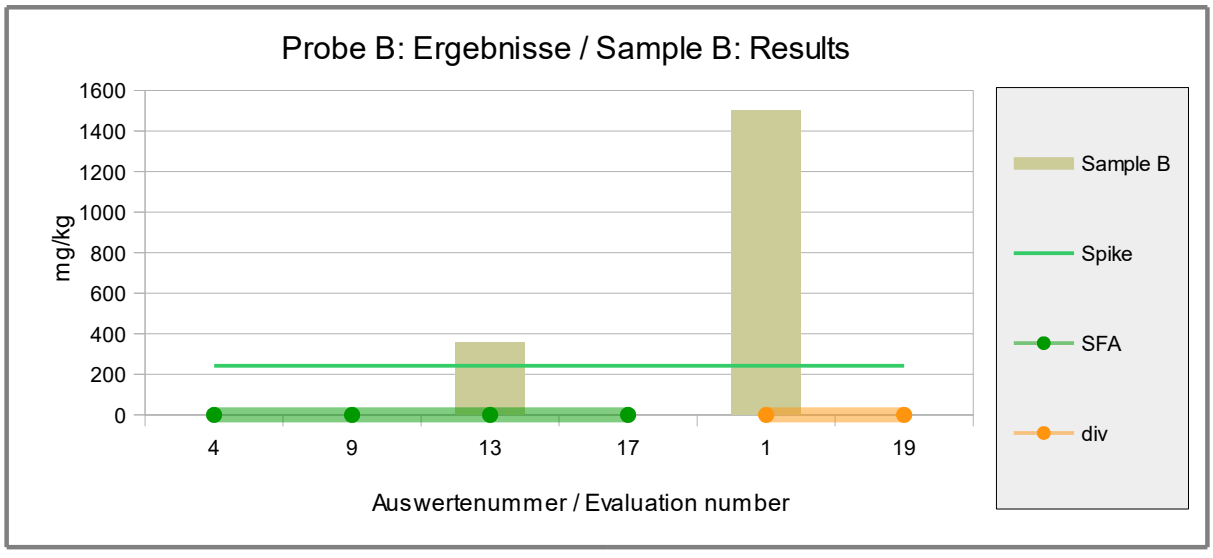


Abb./Fig. 5: PCR Results Crustaceae (fresh)
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Evaluation number	Crustaceae	Crustaceae	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive			SFA	
9	positive			SFA	
13	positive	530		SFA	
17	positive			SFA	
1	positive	7000		div	
19	positive			div	

° calculation see p. 19

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

No quantitative evaluation was done, because there were to few quantitative results.

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

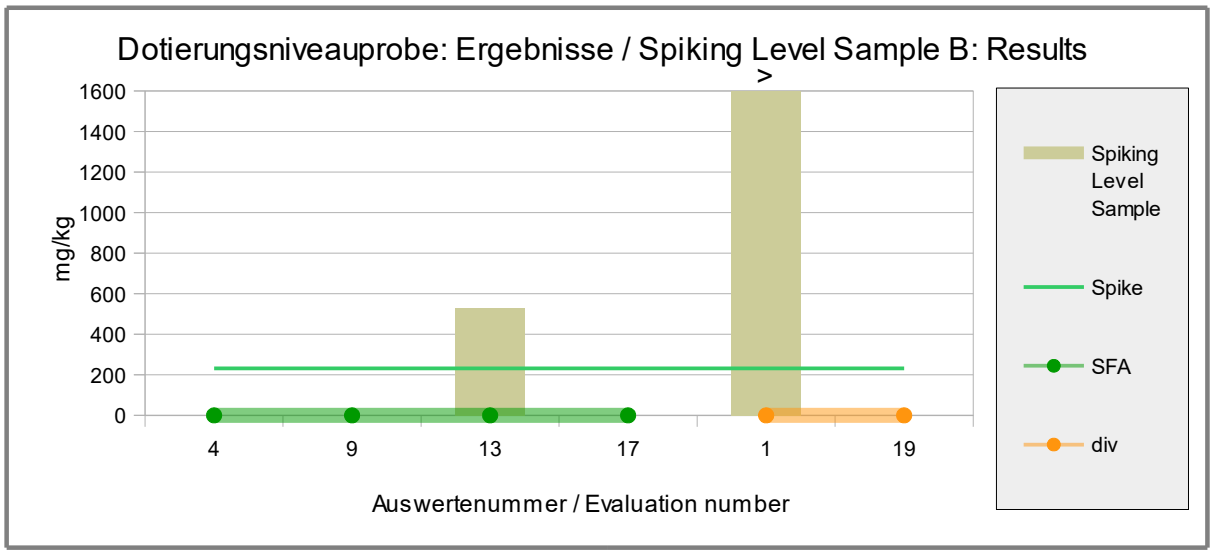


Abb./Fig. 6: PCR Results Crustaceae (fresh)
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Crustaceae (fresh): 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}]		
4							SFA	
9							SFA	
13	530	229	5,2	356	154	2,2	SFA	
17							SFA	
1	7000	3030	117	1500	649	22	div	
19							div	

RA**	50-150 %	RA**	50-150 %
Anzahl im AB	0	Anzahl im AB	0
Prozent im AB	0	Prozent im AB	0

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

* Recovery rate 100% relative size: crustaceae, fresh

** Range of acceptance of AOAC for allergen ELISAS

Comments:
Two participant submitted quantitative results by PCR. All recovery rates for the spiking level sample and the sample B were above the range of the AOAC-recommendation of 50-150%.

4.2 Proficiency Test Peanut

4.2.1 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	< 1.00	positive	27,9	2/2 (100%)	AQ	
16	negative	<1	positive	31,3	2/2 (100%)	AQ	
18	negative	<1	positive	34,2	2/2 (100%)	AQ	
20	negative	<LOD	positive	30,5	2/2 (100%)	AQ	
22	negative	0	positive	58,0	2/2 (100%)	BF	
19	negative	<NG	positive	17,0	2/2 (100%)	IFP	
3	negative		positive	26,4	2/2 (100%)	IL	
17	negative		positive	294	2/2 (100%)	IL	
5	negative	<0,9	positive	18,5	2/2 (100%)	MI	Result converted °
6	negative	<2,5	positive	38,2	2/2 (100%)	RS	
7	negative	<2,5	positive	28,8	2/2 (100%)	RS-F	
9	negative		positive	33,0	2/2 (100%)	RS-F	
12	negative	<2,5	positive	21,0	2/2 (100%)	RS-F	
13	negative	<1	positive	27,4	2/2 (100%)	RS-F	
14	negative	<2,5	positive	28,0	2/2 (100%)	RS-F	
21	negative	0	positive	19,0	2/2 (100%)	SP	

° calculation see p. 19

	Sample A		Sample B	
Number positive	0		16	
Number negative	16		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positiv	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IFP = ELISA-Fast, ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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	27,9	-0,03		AQ	
16	31,3	0,46		AQ	
18	34,2	0,87		AQ	
20	30,5	0,35		AQ	
22	58,0	4,3		BF	
19	17,0	-1,6		IFP	
3	26,4	-0,24		IL	
17	294	38		IL	
5	18,5	-1,4		MI	Result converted °
6	38,2	1,4		RS	
7	28,8	0,11	0,17	RS-F	
9	33,0	0,70	0,78	RS-F	
12	21,0	-1,0	-0,96	RS-F	
13	27,4	-0,10	-0,04	RS-F	
14	28,0	-0,01	0,05	RS-F	
21	19,0	-1,3		SP	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IFP = ELISA-Fast, ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

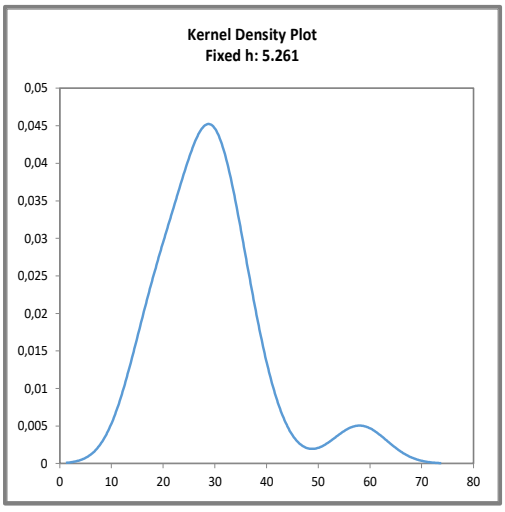


Abb. / Fig. 7:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 around 30 mg/kg with a small side peak at approx. 58 mg/kg due to a value outside the target range (outlier at approx. 300 mg/kg not shown).

Characteristics: Quantitative evaluation ELISA Peanut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Methode RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	15°	5
Number of outliers	1	0
Mean	29,3	27,6
Median	28,0	28,0
Robust Mean (X_{pt})	**28,1**	**27,6**
Robust standard deviation (S*)	**7,83**	**4,89**
Target range:		
Target standard deviation σ_{pt}	**7,01**	**6,91**
lower limit of target range	**14,0**	**13,8**
upper limit of target range	**42,1**	**41,5**
Quotient S/σ_{pt}*	*1,1*	*0,71*
Standard uncertainty $U_{(Xpt)}$	*2,53*	*2,73*
Results in the target range	*14*	*5*
Percent in the target range	*93*	*100*

° *without result no. 17 (outlier excluded)*

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

Comments to the statistical characteristics and assigned values:

The kernel density showed almost a symmetrical distribution of results.

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 218% and 214% of the spiking level of peanut to sample B and thus above the range of the recommendations for the applied methods (s. 3.4.3 and p.43 "Recovery rates ELISA for peanut").

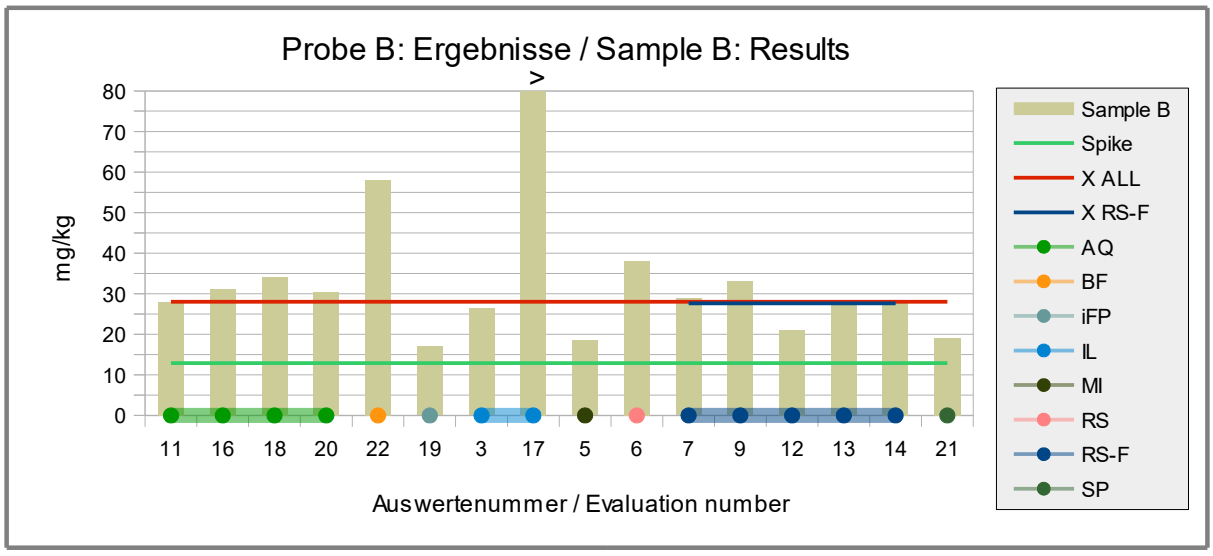


Abb./Fig. 8: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

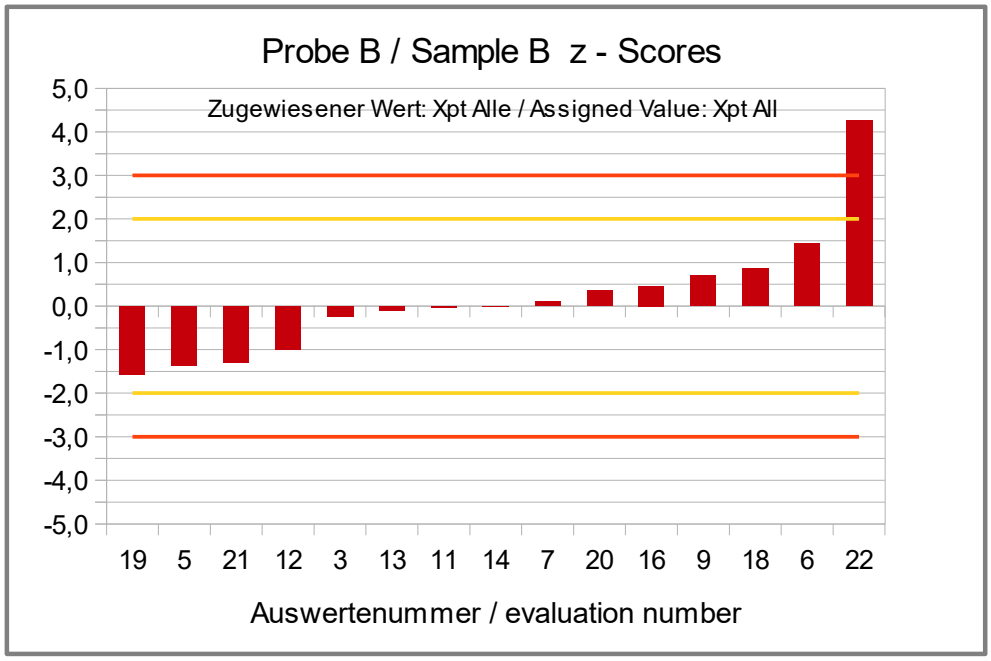


Abb./Fig. 9:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

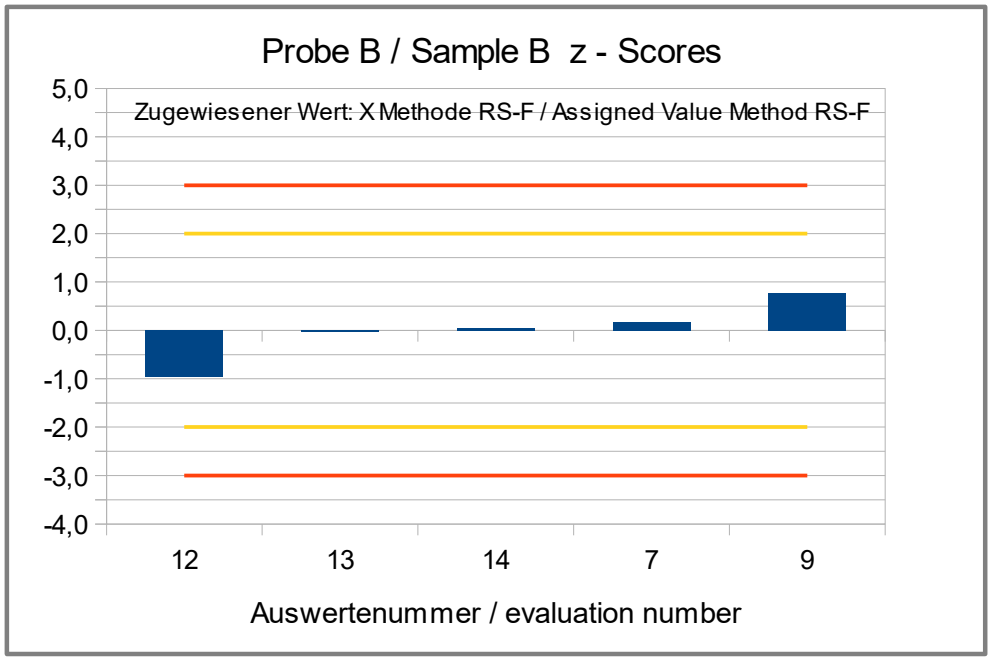


Abb./Fig. 10:
z-Scores (ELISA Results Peanut) Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	pos/neg	[mg/kg]				
11	positive	38,8	0,19		AQ	
16	positive	47,5	1,13		AQ	
18	positive	41,9	0,53		AQ	
20	positive	41,0	0,43		AQ	
22	positive	104	7,2		BF	
19	positive	16,0	-2,3		IFP	
3	positive	27,0	-1,08		IL	
17	positive	335			IL	Outlier excluded
5	positive	29,3	-0,8		MI	Result converted °
6	positive	82,0	4,9		RS	
7	positive	36,5	-0,06	0,12	RS-F	
9	positive	40,0	0,32	0,52	RS-F	
12	positive	15,5	-2,3	-2,25	RS-F	
13	positive	41,7	0,51	0,71	RS-F	
14	positive	35,0	-0,22	-0,05	RS-F	
21	positive	28,0	-1,0		SP	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IFP = ELISA-Fast, ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

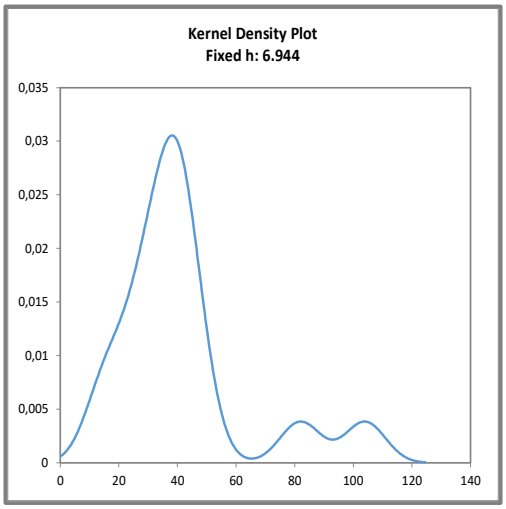


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution around the robust mean of 37 mg/kg with three small side peaks due to two results outside the target range (outlier at approx. 300 mg/kg not shown).

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	15°	5
Number of outliers	1	0
Mean	41,6	33,7
Median	38,8	36,5
Robust Mean (X_{pt})	**37,0**	**35,4**
Robust standard deviation (S*)	**14,4**	**7,9**
Target range:		
Target standard deviation σ_{pt}	**9,26**	**8,86**
lower limit of target range	**18,5**	**17,7**
upper limit of target range	**55,5**	**53,1**
Quotient S/σ_{pt}*	*1,6*	*0,89*
Standard uncertainty $U_{(Xpt)}$	*4,66*	*4,43*
Results in the target range	*11*	*4*
Percent in the target range	*73*	*80*

° *without result no. 17 (excluded)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results.

The 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 246% and 235% of the spiking level of peanut to the spiking level sample and thus above the range of the recommendations for the applied methods (s. 3.4.3 and p.43 "Recovery rates ELISA for peanut").

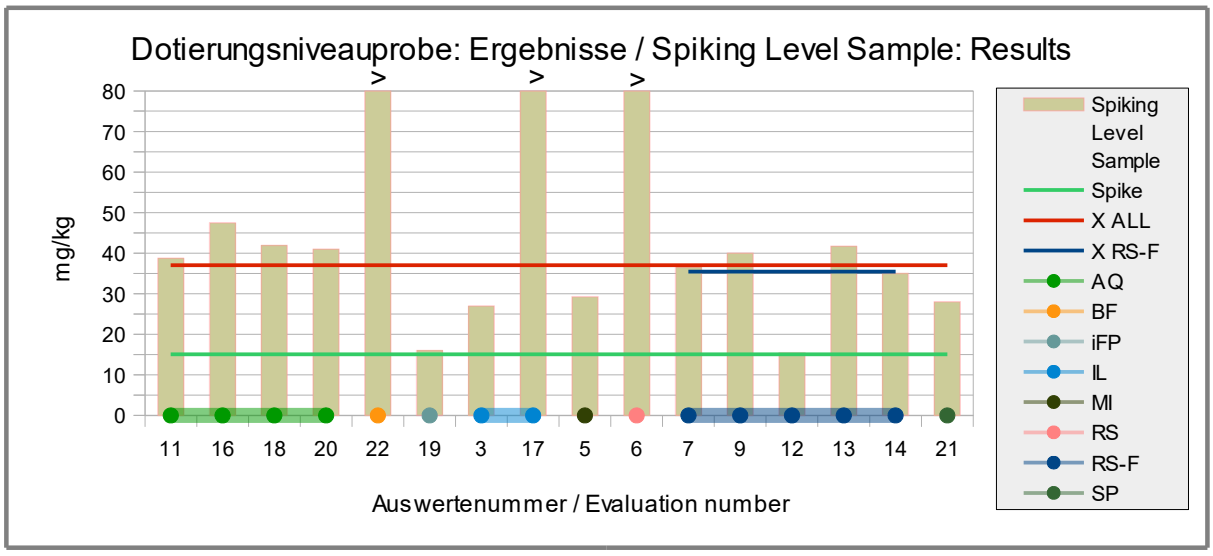


Abb./Fig. 12: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

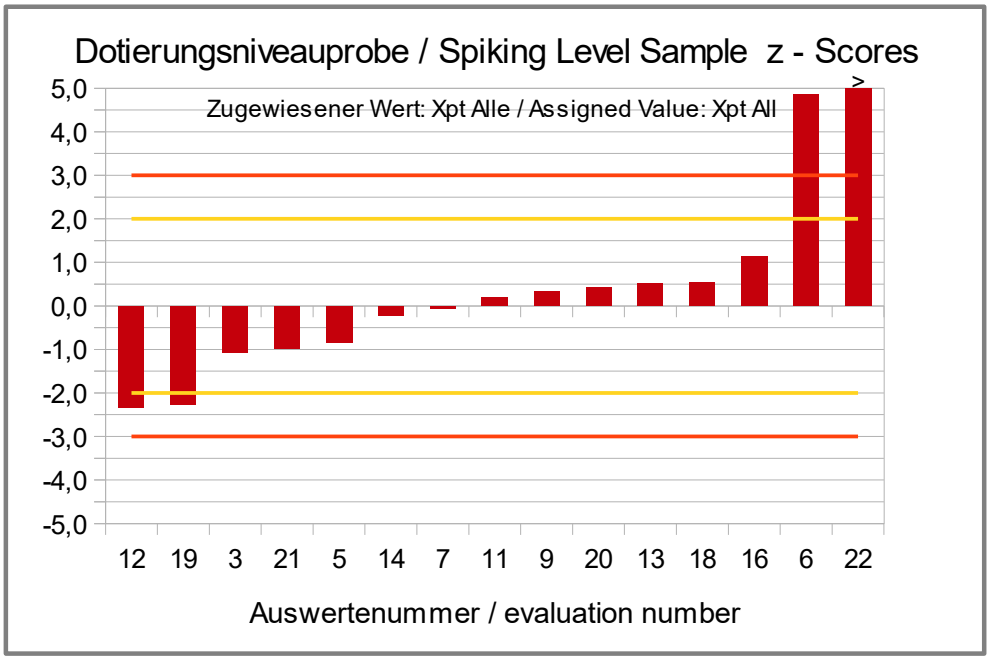


Abb./Fig. 13:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

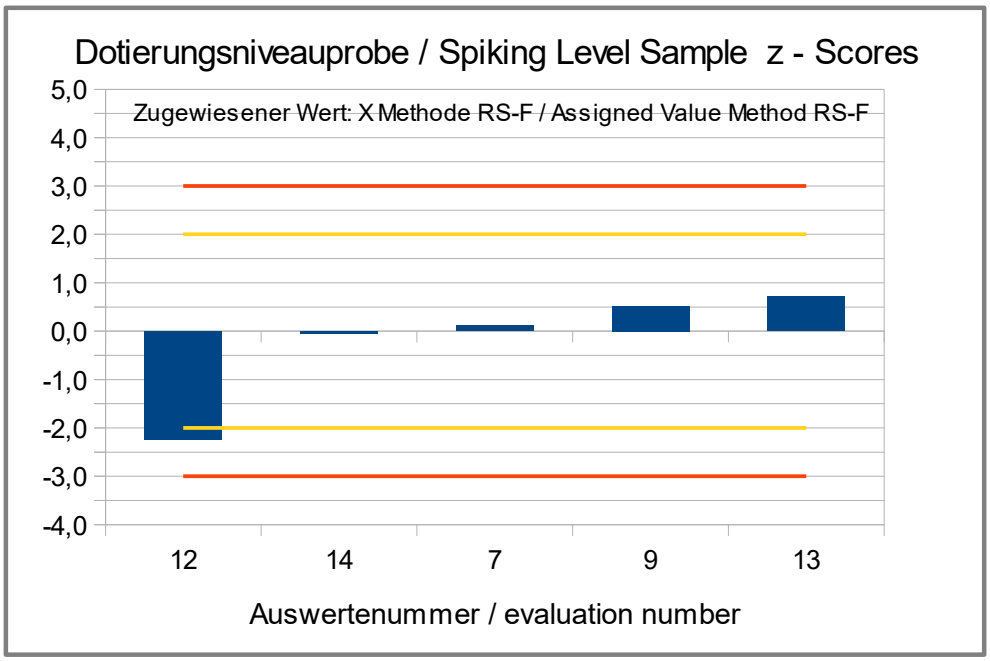


Abb./Fig. 14:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

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	38,8	257	6,3	27,9	216	4,6	AQ	
16	47,5	315	8,6	31,3	242	5,7	AQ	
18	41,9	278	7,1	34,2	265	6,6	AQ	
20	41,0	272	6,9	30,5	237	5,5	AQ	
22	104	691	24	58,0	450	14	BF	
19	16,0	**106**	0,25	17,0	**132**	1,3	IFP	
3	27,0	179	3,2	26,4	205	4,2	IL	
17	335	2224	85	294	2279	87	IL	
5	29,3	195	3,8	18,5	**143**	1,7	MI	Result converted °
6	82,0	544	17,8	38,2	296	7,8	RS	
7	36,5	242	5,7	28,8	223	4,9	RS-F	
9	40,0	266	6,6	33,0	256	6,2	RS-F	
12	15,5	**103**	0,12	21,0	163	2,5	RS-F	
13	41,7	277	7,1	27,4	212	4,5	RS-F	
14	35,0	232	5,3	28,0	217	4,7	RS-F	
21	28,0	186	3,4	19,0	**147**	1,9	SP	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	2	Number in RA	3
Percent in RA	13	Percent in RA	19

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

** Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IFP = ELISA-Fast, ifp

IL = Immunolab

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

For the spiking level sample, almost all recovery rates obtained by ELISA methods were well above the AOAC requirement of 50-150% (exception results no. 12 and 19). Similarly, for the processed spiked food matrix sample B, only three recovery rates (results no. 5, 19 and 21) were in the range of 50-150%.

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

4.2.2 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		
5	negative		positive		2/2 (100%)	ASU	
9	negative		positive		2/2 (100%)	CEN	
17	negative		positive		2/2 (100%)	GI	
13	negative	<1	positive	22,17	2/2 (100%)	SFA	
1	negative		positive	610	2/2 (100%)	div	
19	negative		positive	15	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		6	
Number negative	6		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
CEN = Euroäische Norm/ European Comittee for standardization
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

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

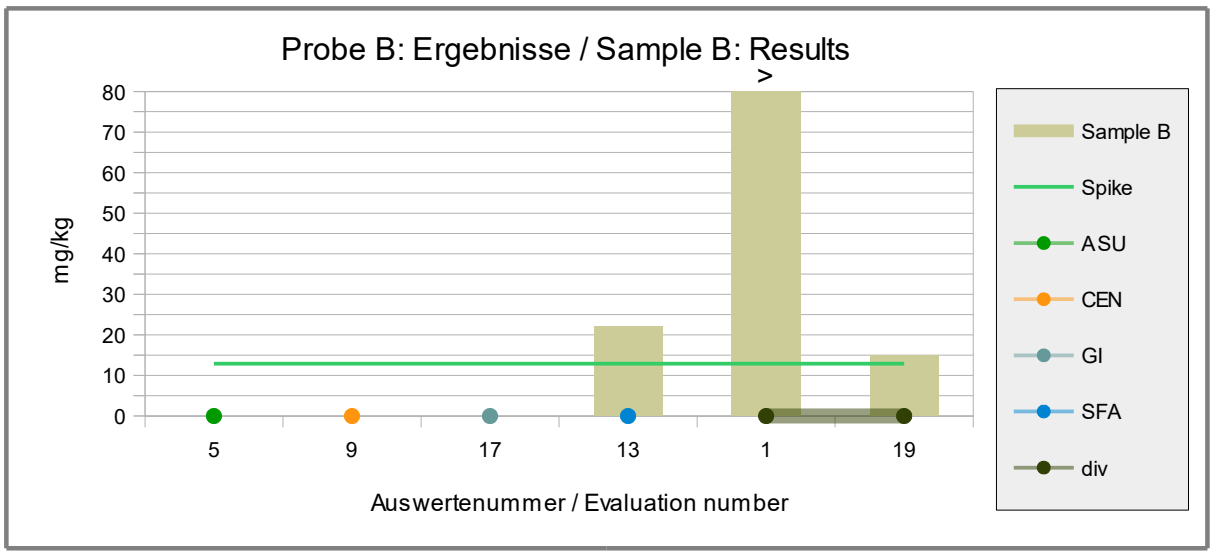


Abb./Fig. 15: PCR Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Evaluation number	Peanut	Peanut	z-Score Xpt_{RS}	Method	Remarks
	pos/neg	[mg/kg]			
5	positive			ASU	
9	positive			CEN	
17	positive			GI	
13	positive	23,3		SFA	
1	positive	1400		div	
19	positive	15,0		div	

Methods:
ASU = ASU §64 Methode/method
CEN = Euroäische Norm/ European Comittee for standardization
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div = not indicated / other method

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

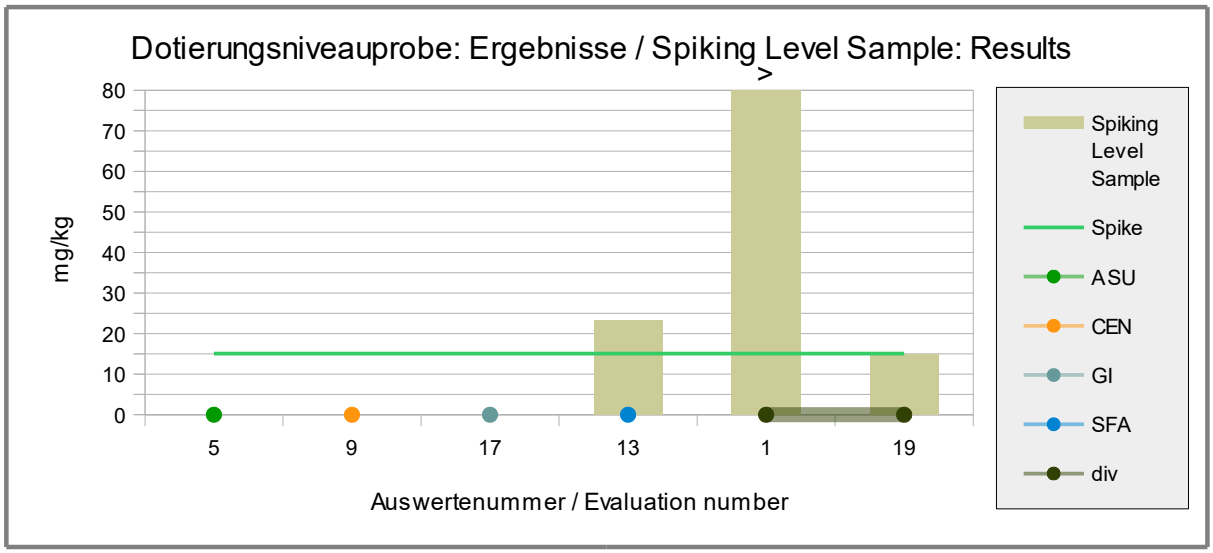


Abb./Fig. 16: PCR Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR 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}]		
5							ASU	
9							CEN	
17							GI	
13	23,3	154	2,2	22,2	172	2,9	SFA	
1	1400	9296	368	610	4729	185	div	
19	15,0	**100**	0,0	15,0	**116**	0,65	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	33	Percent in RA	33

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

CEN = Euroäische Norm/ European Comittee for standardization

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Three participants submitted quantitative results by PCR. One participant obtained recovery rates for the spiking level sample and the sample B within the range of the AOAC recommendation of 50-150%.

4.3 Proficiency Test Coconut

4.3.1 ELISA Results: Coconut (as Coconut flour)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
4	negative	<1	positive	>40	2/2 (100%)	BF	
11	negative	< 2.00	positive	313	2/2 (100%)	BF	
22	negative	0	positive	659	2/2 (100%)	BF	
13	negative	<1,1	positive	112	2/2 (100%)	DE	Result converted °
20	negative	<LOD	positive	55,4	2/2 (100%)	ET	
19	negative	<NG	positive	121	2/2 (100%)	IFP	
9	negative		positive	109	2/2 (100%)	IL	Result converted °
5	negative	<1,1	positive	88,3	2/2 (100%)	SP	Result converted °
21	negative	0	positive	92,2	2/2 (100%)	SP	Result converted °

° calculation see p. 19

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies
DE = Demeditec ELISA
ET = Elution Technologies ELISA Kit
IFP = ELISA-Fast, ifp
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Coconut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4	>40		BF	
11	313		BF	Result excluded
22	659		BF	Result excluded
13	112	0,62	DE	Result converted °
20	55,4	-1,7	ET	
19	121	1,0	IFP	
9	109	0,49	IL	Result converted °
5	88,3	-0,36	SP	Result converted °
21	92,2	-0,20	SP	Result converted °

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
DE = Demeditec ELISA
ET = Elution Technologies ELISA Kit
IFP = ELISA-Fast, ifp
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

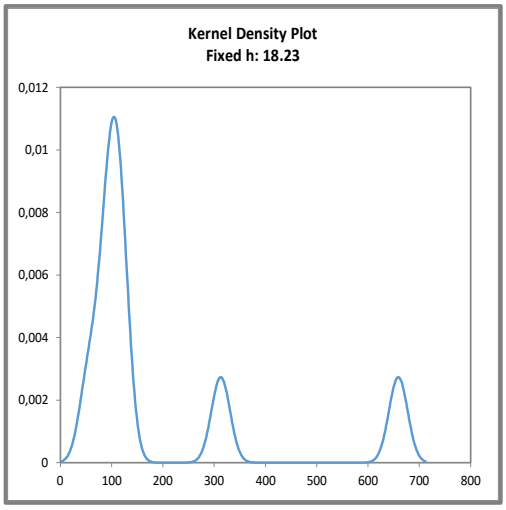


Abb. / Fig. 17:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimate shows almost a symmetrical distribution of results with a maximum at approx. 100 mg/kg and two smaller peaks due to two results of method BF.

Characteristics: Quantitative evaluation ELISA Coconut

Due to the limited number of results, the following evaluation is only given for information:

Sample B

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	6°
Number of outliers	2
Mean	96,4
Median	101
Robust Mean (X_{pt})	**97,1**
Robust standard deviation (S*)	**24,9**
Target range:	
Target standard deviation σ_{pt}	**24,3**
lower limit of target range	**48,6**
upper limit of target range	**146**
Quotient S/σ_{pt}*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*12,7*
Results in the target range	*6*
Percent in the target range	*100*

° *without results no. 11 and 22 (outlier excluded)*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results with two higher results. These results were therefore excluded from the statistical evaluation.

The evaluation of all methods showed a normal variability of results, with a quotient 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 limited, because there were only a few results for some methods. The target value does not apply to the single methods.*

The robust mean of the evaluation was 117% of the spiking level of coconut flour to sample B and thus within range of the recommendations for the applied methods (s. 3.4.3 and p.53 "Recovery rates ELISA for Coconut").

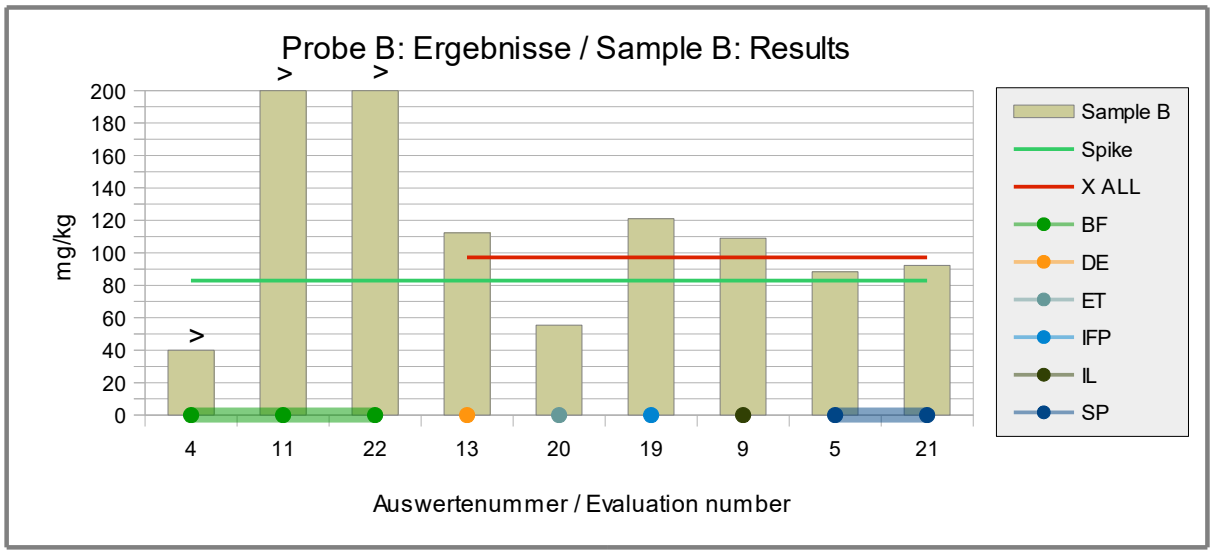


Abb./Fig. 18: ELISA Results Coconut
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

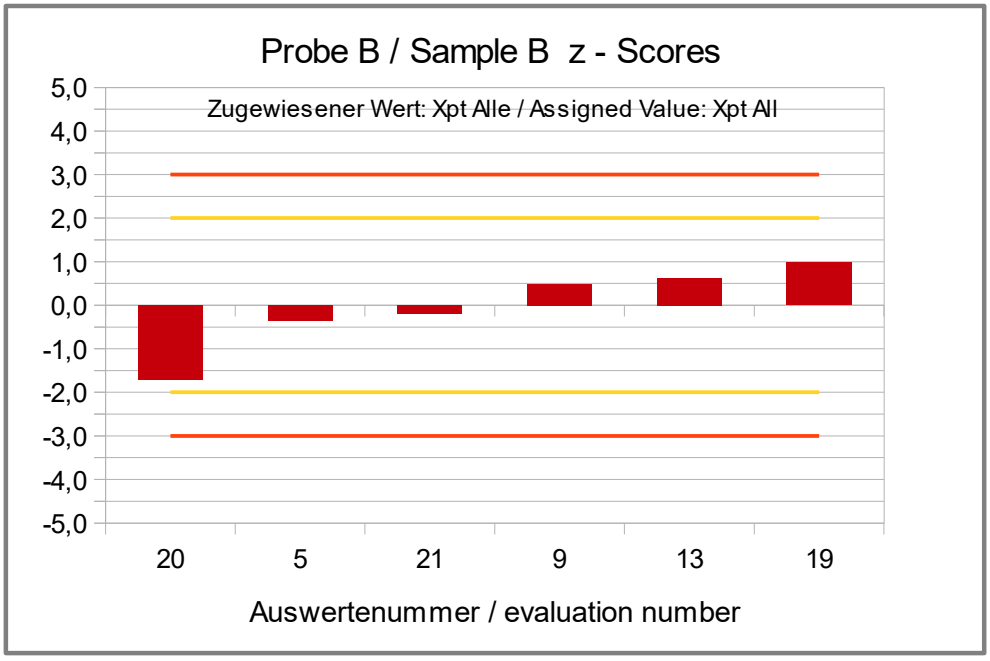


Abb./Fig. 19:
z-Scores (ELISA Results Coconut)
Assigned value robust mean of all results

Quantitative valuation of results: Spiking Level Sample

Evaluation number	Coconut	Coconut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive	>40		BF	
11	positive	174	1,3	BF	
22	positive	428	9,1	BF	
13	positive	98,0	-1,0	DE	Result converted °
20	positive	146	0,45	ET	
19	positive	197	2,0	IFP	
9	positive	83,9	-1,4	IL	Result converted °
5	positive	116	-0,46	SP	Result converted °
21	positive	78,9	-1,6	SP	Result converted °

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
DE = Demeditec ELISA
ET = Elution Technologies ELISA Kit
IFP = ELISA-Fast, ifp
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

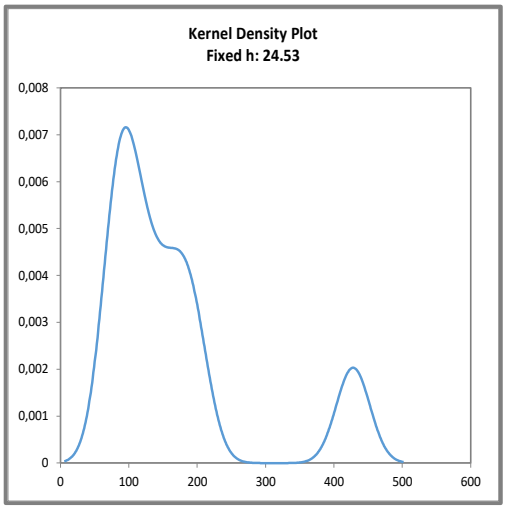


Abb. / Fig. 20:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a main peak with at approx. 100 mg/kg with a shoulder and a smaller side peak at approx. 428 mg/kg, due to a single value outside the target range (method BF).

Characteristics: Quantitative evaluation ELISA Coconut

Due to the limited number of results, the following evaluation is only given for information:

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	8
Number of outliers	0
Mean	165
Robust Mean	142
Median (X_{pt})	**131**
Robust standard deviation (S*)	**66,3**
Target range:	
Target standard deviation σ_{pt}	**32,7**
lower limit of target range	**65,4**
upper limit of target range	**196**
Quotient S/σ_{pt}*	*2,0*
Standard uncertainty $U_{(Xpt)}$	*29,3*
Results in the target range	*7*
Percent in the target range	*88*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution with a shoulder and one higher result. No results were excluded, because there was no clear method-dependent trend.

The evaluation of all methods showed a normal variability of results. The quotient S/σ_{pt} was at 2,0. The robust standard deviation is 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 limited, because there were only a few results for some methods. The target value does not apply to the single methods.*

The robust mean of the evaluation was 175% of the spiking level of coconut flour to the spiking level sample and thus within the recommendations for the applied methods (s. 3.4.3 and p.53 "Recovery rates ELISA for Coconut").

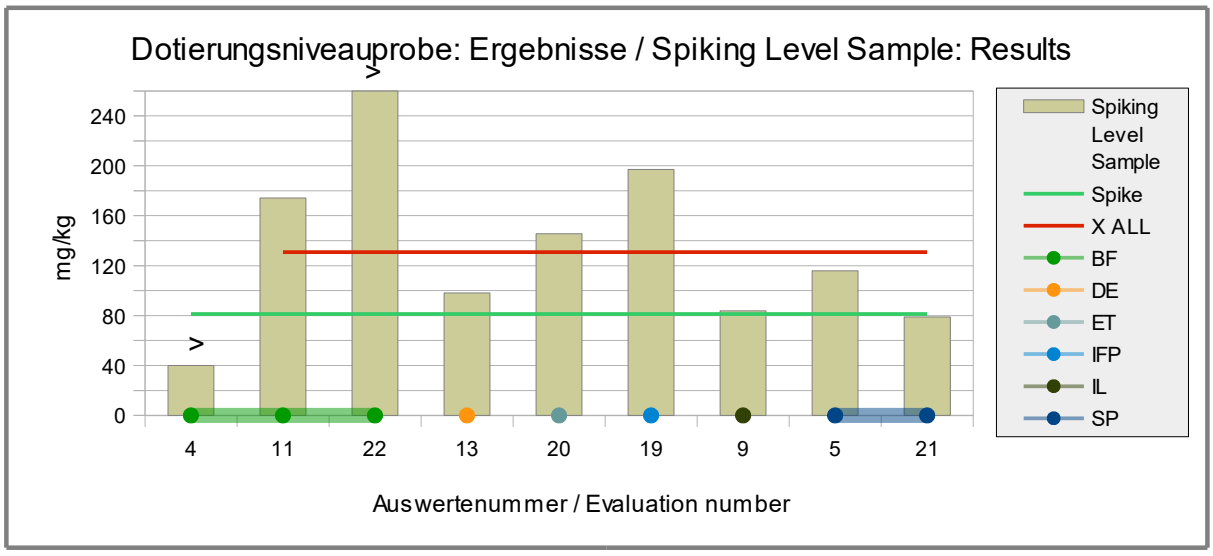


Abb./Fig. 21: ELISA Results Coconut
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

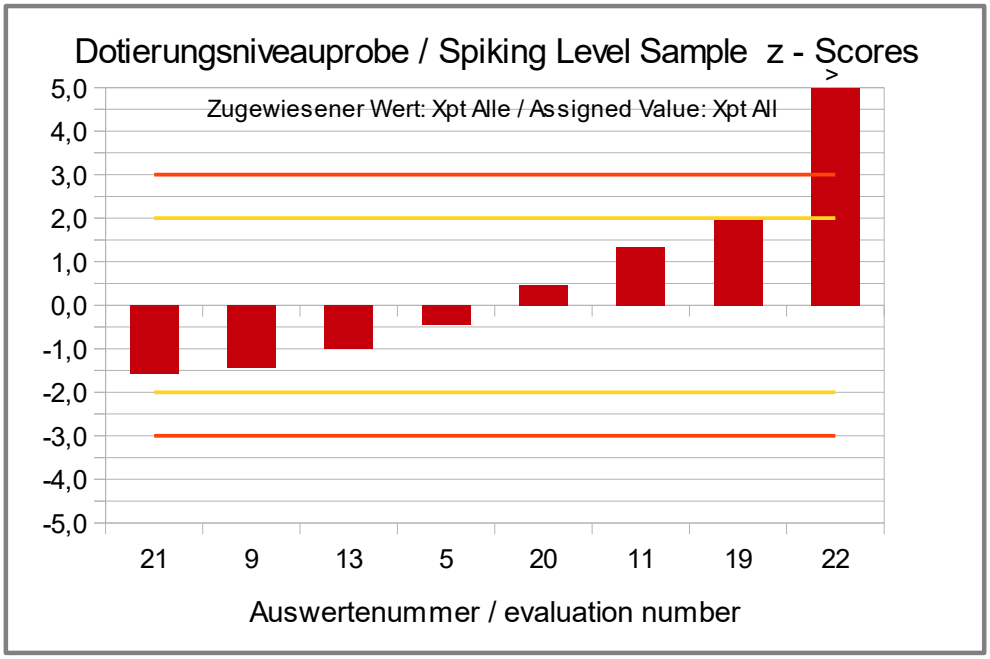


Abb./Fig. 22:
z-Scores (ELISA Results Coconut)
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA for Coconut: 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}]		
4	>40			>40			BF	
11	174	215	4,6	313	377	11	BF	
22	428	527	17	659	795	28	BF	
13	98,0	**121**	0,83	112	**135**	1,4	DE	Result converted °
20	146	179	3,2	55,4	**67**	-1,3	ET	
19	197	243	5,7	121	**146**	1,8	IFP	
9	83,9	**103**	0,13	109	**131**	1,3	IL	Result converted °
5	116	**143**	1,7	88,3	**107**	0,26	SP	Result converted °
21	78,9	**97**	-0,11	92,2	**111**	0,45	SP	Result converted °

° Calculation page 19

RA**	50-150 %	RA**	50-150 %
Number in RA	4	Number in RA	6
Percent in RA	50	Percent in RA	75

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

** Range of acceptance of AOAC for allergen ELISAs

Methods:

BF = MonoTrace ELISA, BioFront Technologies
DE = Demeditec ELISA
ET = Elution Technologies ELISA Kit
IFP = ELISA-Fast, ifp
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

Comments:

50% (4) of the participants obtained a recovery rate by ELISA methods for the spiking level sample within the range of the AOAC-recommendation of 50-150%. For the processed, spiked food matrix sample B, 75% (6) participants were in the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.3.2 PCR Results: Coconut

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		
1	negative		positive	210	2/2 (100%)	div	
19	negative		positive	110	2/2 (100%)	div	

	Probe A		Probe B	
Number positive	0		2	
Number negative	2		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methoden:
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

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

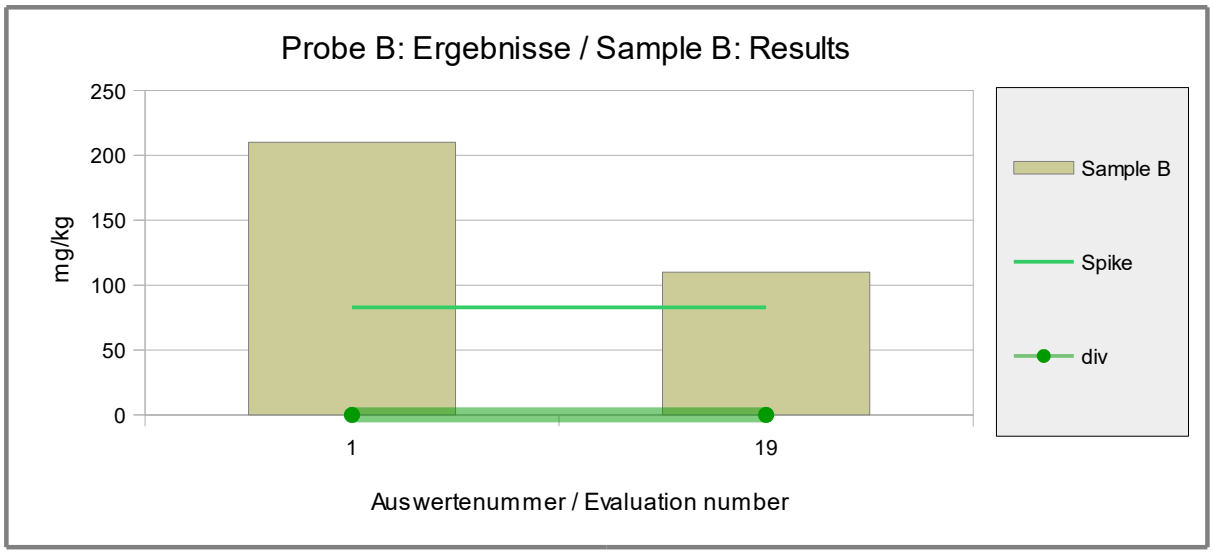


Abb./Fig. 23: PCR Results Coconut
green line = Spiking level
round symbols = Applied methods (see legend)

Qualitative valuation PCR: Spiking Level Sample

Evaluation number	Coconut	Coconut	z-Score Xpt	Method	Remarks
	pos/neg	[mg/kg]			
1	positive	360		div	
19	positive	180		div	

Number positive	2
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methoden:
div = not indicated / other method

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

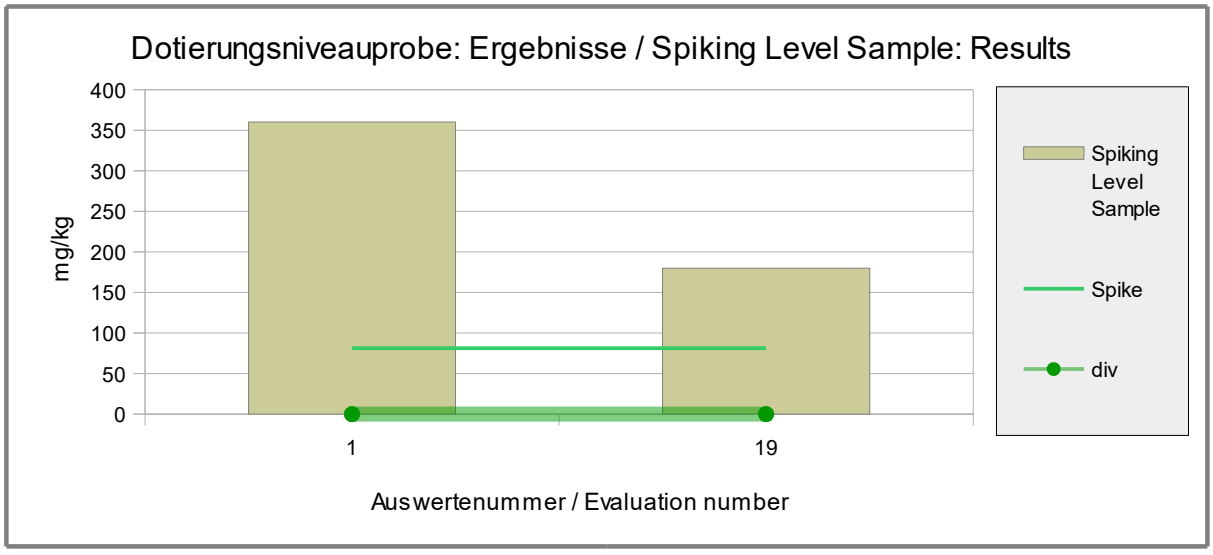


Abb./Fig. 24: PCR Results Coconut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Coconut:
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}]		
1	360	443	14	210	253	6,1	div	
19	180	222	4,8	110	**133**	1,3	div	

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

Methods:
div = not indicated / other method

* Recovery rate 100% relative size: coconut, s. page 4

** Range of acceptance of AOAC for allergen ELISAS

Comments:
For the spiking level sample, both recovery rates obtained by PCR methods were well above the AOAC requirement of 50-150%. For the processed spiked food matrix sample B, one participant was within the acceptance range.
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 Peanut: Xpt (div. Methods)		ELISA Peanut: Xpt (Method: RS-F)	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
3	-0,24	-1,1		
5	-1,4	-0,8		
6	1,4	4,9		
7	0,11	-0,06	0,17	0,12
9	0,70	0,32	0,78	0,52
11	-0,03	0,19		
12	-1,0	-2,3	-0,96	-2,2
13	-0,10	0,51	-0,04	0,71
14	-0,01	-0,22	0,05	-0,05
16	0,46	1,1		
17				
18	0,87	0,53		
19	-1,6	-2,3		
20	0,35	0,43		
21	-1,3	-1,0		
22	4,3	7,2		

Evaluation number	ELISA Coconut: Xpt (div. Methods)	
	Sample B	Sp. Level Sample
4		
5	-0,36	-0,46
9	0,49	-1,4
11		1,3
13	0,62	-1,0
19	1,0	2,0
20	-1,7	0,45
21	-0,20	-1,6
22		9,1

Comments: For the parameter Crustaceae, no statistical evaluation and calculation of z-scores was conducted.

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 Crustaceae Protein		ELISA Peanut		ELISA Coconut	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
1						
2	-3,5	-3,5				
3	-3,1	-3,0	4,2	3,2		
4						
5	-3,1	-3,1	1,7	3,8	0,26	1,7
6			7,8	18		
7	34	38	4,9	5,7		
8	6,8	8,1				
9			6,2	6,6	1,3	0,13
10	-2,6	-2,6				
11			4,6	6,3	11	4,6
12	-3,6	-3,8	2,5	0,12		
13	30	38	4,5	7,1	1,4	0,83
14	4,1	5,2	4,7	5,3		
15	-2,7	-2,7				
16	-3,2	-3,1	5,7	8,6		
17			87	85		
18	-3,8	-3,9	6,6	7,1		
19			1,3	0,25	1,8	5,7
20	-3,9	-3,9	5,5	6,9	-1,3	3,2
21	-2,9	-2,3	1,9	3,4	0,45	-0,11
22	8,2	-0,17	14	24	28	17

Evaluation number	PCR Crustaceae		PCR Peanut		PCR Coconut	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
1	22	117	185	368	6	14
4						
5						
9						
13			2,9	2,2		
17						
19	2,2	5,2	0,65	0,0	1,3	4,8

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: Crustaceae

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	10	15.12.20	negative	<0.1	positive	16,3	positive	15,5	0,1	0,1	53,1	Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AQ	16	05.01.21	-	<0.2	-	9,09	-	10,2	0,2	0,2	45,45	Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AQ	18	04.01.21	negative	<0.02	positive	1,75	positive	1,45		0,02	48,3	Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AQ	20	17. Nov	-	<LOD	-	3,32	-	4,15	0,0045	0,1		Crustaceae, fresh	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AS	19	06.01.21	negative		positive		positive		10			Crustaceae, fresh	AgraStrip® Crustacea (COKAL2210AS) RomerLabs
BF	4		negative	<1	positive	>40	positive	>40		1	32,2	Crustaceae, fresh	MonoTrace Crustacea ELISA kit, BioFront Technologies
BF	22	08.01.21	negative	0	positive	742	positive	221	0,07	1		Crustaceae, fresh	Selection Crustaceae-Kits:
ES	12	13.11.2020	negative	<0,05	positive	0,79	positive	0,53	0,05	0,05	50	Tropomyosin protein	ELISA Systems Crustacean ESCRURD-48
IL	3	03.12.20	negative		positive	1,97	positive	2,07	0,02	0,02		Crustaceaeprotein	Immunolab Crustaceans (Tropomyosin) ELISA
RS-F	7	17.12.20	negative	<20	positive	500	positive	520		20		Crustaceae, dried	Ridascreen® FAST Crustacean R7312, R-Biopharm
RS-F	8	30.11.20	-	<20ppm	-	122,45	-	131,44	20			Crustacea protein	Ridascreen® FAST Crustacean R7312, R-Biopharm
RS-F	13	27.11.20	negative	<20	positive	388,72	positive	450,72	20	20		Crustaceae, fresh	Ridascreen® FAST Crustacean R7312, R-Biopharm
RS-F	14	27.11.20	negative	<20	positive	490	positive	530	2	20		Crustaceae, fresh	Ridascreen® FAST Crustacean R7312, R-Biopharm
SP	5	13.11.20	negative	<0,02	positive	2	positive	1,9	0,01	0,02		Tropomyosin in crustaceae	Eurofins SensiSpec Crustaceans (Tropomyosin) ELISA Kit
SP	15	16.12.20	-	<0,001	-	2,9	-	2,9	0,001	0,02	47	Crustacea protein (TROPOMYOSIN)	Eurofins SensiSpec Crustaceans (Tropomyosin) ELISA Kit
SP	21	19.11.20	negative	0	positive	2,59	positive	3,6	0,0009	0,02		Tropomyosin	Eurofins SensiSpec Crustaceans (Tropomyosin) ELISA Kit
VT	2	24.11.20	negative	<2.5	positive	24	positive	22,4		2,5		Crustaceae, fresh	Veratox Crustacea, Neogen

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

Continuation ELISA Crustaceae:

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	10	crustacean tropomyosin	19mL ES pre-heated / 15 minutes / 40 degrees	yes	
AQ	16			Yes	
AQ	18			Yes	
AQ	20				
AS	19	polyclonal		PV-96-IF-PF-29 : 2019-07 (a)	quick test
BF	4			yes	
BF	22	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 42C	no	
ES	12			yes	
IL	3				
RS-F	7			yes	
RS-F	8				
RS-F	13	As Per Kit Instructions	As Per kit instructions	Yes	
RS-F	14	Crustacean proteins, mainly tropomyosin		yes	
SP	5	detects Crustacean tropomyosin	As Per kit instructions	yes	HU 0030006/HU 0030030
SP	15	agains tropomyosin	1 g sample/20 mL extraction buffer ->15' at 40°C -> 10' at 2000xg-> 100 ul supernatant are analysed	yes	
SP	21				
VT	2			yes	

5.1.2 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
AQ	11	16/11	-	< 1.00	-	27,88	-	38,76	0,1	1		Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
AQ	16	05.01.21	-	<1	-	31,26	-	47,45	1	1	31,79	Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
AQ	18	21.12.20	negative	<1	positive	34,15	positive	41,92		1	48,8	Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
AQ	20	23. Dez	-	<LOD	-	30,53	-	41	0,1	1		Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
BF	22	08.01.21	negative	0	positive	58	positive	104	0,12	1		Peanut	Selection Peanut-Kits:
IFP	19	14.12.20	negative	<NG	positiv	17	positive	16	0,5	1	19,4	Peanut	ELISAFast® Erdnuss
IL	3	19.11.20	negative		positiv	26,4	positive	27	1	1		Peanut	Immunolab Peanut ELISA
IL	17	05.01.21	negative		positiv	294	positiv	335		1		Peanut	Immunolab Peanut ELISA
MI	5	12.11.20	negative	<0,2	positiv	4,3	positive	6,8	0,2	0,2		Peanut protein	Peanut ELISA Kit enhanced Morinaga
RS	6	22.12.20	-	<2,5	-	38,18	-	81,95	0,13	2,5	20	Peanut	Ridascreen Peanut (R6201), r-Biopharm
RS-F	7	17.12.20	negative	<2,5	positiv	28,8	positive	36,5		2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	9		negative		positive	33	positive	40	0,6	2		Peanut	AOAC RI PTM 030404 RIDASCREEN - FAST Peanut Art. No. R6202
RS-F	12	13.11.2020	negative	<2,5	positive	21	positive	15,5	1	2,5	50	Peanut	Veratox Peanut, Neogen
RS-F	13	27.11.20	negative	<1	positive	27,37	positive	41,73	1	1		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	09.12.20	negative	<2,5	positive	28	positive	35	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
SP	21	19.11.20	negative	0	positive	19	positive	28	0,1	1		Peanut	Eurofins SensiSpec Peanut ELISA Kit

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

Continuation ELISA Peanut:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	11			Yes	
AQ	16			Yes	
AQ	18			Yes	
AQ	20				
BF	22	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 60C	no	
IFP	19	polyclonal		ASU L 00.00-69 : 2003-12 (a)	Sandw ich ELISA
IL	3				
IL	17			yes	
MI	5	detecs peanut protein	As Per kit instructions	yes	MloBS Test-Combination M2120
RS	6		Extraction w ith extraction buffer diluited at 60°C for 10 minutes. Then, centrifuged at 2500g per 10 minutes. Use the supernatant t.q..	yes	the analyzes on samples b and spike w ere repeated tw ice: the first on as is, the second w ith dilution in the extraction process (factor 1:2).
RS-F	7			yes	
RS-F	9			Yes	
RS-F	12			yes	
RS-F	13	As Per Kit Instructions	As Per kit instructions	Yes	
RS-F	14	Peanut proteins, i.e. Ara h1 and Ara h2		yes	
SP	21				

5.1.3 ELISA: Coconut

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
BF	4		negative	<1	positive	>40	positive	>40		1	19,4	Coconut	MonoTrace Coconut ELISA kit, BioFront Technologies
BF	11	04.12.21	-	< 2.00	-	312,81	-	174,27	0,13	2		Coconut	MonoTrace Coconut ELISA kit, BioFront Technologies
BF	22	08.01.21	negative	0	positive	659	positive	428	0,13	1		Coconut flour	Selection Coconut-Kits:
DE	13	25.02.2021	negative	<2	positive	203,45	positive	177,62	2	2		Coconut	
ET	20	08. Jan	-	<LOD	-	55,4	-	145,6	1	2		Coconut	Elution Technologies ELISA Kit Coconut Protein E-75CNT
IFP	19	06.01.21	negative	<NG	positive	121	positive	197	2	2	20	Coconut flour	ELISAFast® Kokosnuss
IL	9		negative		positive	192	positive	152	0,4	2		Coconut	Immunolab Coconut ELISA
SP	5	17.11.20	negative	<2	positive	160	positive	210	1,5	2		Coconut	Eurofins SensiSpec Coconut ELISA Kit
SP	21	19.11.20	negative	0	positive	167	positive	143	0,4	2		Coconut, fresh	Eurofins SensiSpec Coconut ELISA Kit

* 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	
BF	4			yes	
BF	11			No	
BF	22	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 60C	no	
DE	13	As Per Kit Instructions	As Per kit instructions	Yes	Demeditec Coconut ELISA DECONE01 used
ET	20				
IFP	19	polyclonal		IFP 002822 (ELI-SA) : 2020-07 (a)	Sandwich ELISA
IL	9				
SP	5	Detects coconut protein	As Per kit instructions	Yes	HU0030005/H 0030029
SP	21				

5.1.4 PCR: Crustaceae

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	4		negative	<0,4	positive		positive		0,4			Crustaceae DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9		negative		positive		positive		0,4			Crustaceae DNA	LFOD-TST-SOP-8852 SureFood® ALLERGEN Crustaceans Art. No.: S3612
SFA	13	20.11.20	negative	<1	positive	356,45	positive	529,75	1	1		Crustaceae, fresh	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	07.01.21	negative		positive		positive		0,4			Crustaceae-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
div	1		negative		positive	1500	positive	7000	50	100	30		
div	19		negative		positive		positive		20				

* 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	4			yes	only qualitative test
SFA	9				
SFA	13	As Per Kit Instructions	As Per kit instructions	No	
SFA	17			yes	
div	1	16S rRNA gene in crusta-cean	Wizard/Realtime PCR	no	
div	19	rDNA		PV-20-PCR-PF-104 (a)	Realtime PCR

5.1.5 PCR: Peanut

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
ASU	5	04.12.20	negative		positive		positive		10			Peanut-DNA	ASU §64 Methode/method
CEN	9		negative		positive		positive		10			Peanut DNA	PD CEN/TS 15634-4:2016
GI	17	18.11.20	negative		positive		positive		0,4			Peanut-DNA	GEN-IAL First Allergen
SFA	13	20.11.20	negative	<1	positive	22,17	positive	23,26	1	1		Peanut	Sure Food ALLERGEN, R-Biopharm / Congen
div	1		negative		positive	610	positive	1400	50	100	30		
div	19		negative		positive	15	positive	15	1	1	30	Peanut	

* 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 accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	5		CTAB / Proteinas K / Rnase A / Promega Maxwell / Real-time PCR / 45 Zyklen	yes	§ 64 LFGB L 00.00-169:2019-07
CEN	9				
GI	17			yes	
SFA	13	As Per Kit Instructions	As Per kit instructions	Yes	
div	1	Arachis hypogaea, allergen II gene	Wizard/Realtime PCR	yes	
div	19	allergen II gene		PV-28-PCR-PF-5 (a)	Realtime PCR

5.1.6 PCR: Coconut

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
div	1		negative		positive	210	positive	360	50	100	30		
div	19		negative		positive	110	positive	180	1	1	30	Coconut	

* 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 accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
div	1	Cocos nucifera partial mRNA for actin (act gene)	Wizard/Realtime PCR	no	
div	19	rRNA gene		PV-28-PCR-PF-295 : 2014-11 (a)	Realtime PCR

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL07 Sample B

Weight whole sample	2,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	52	20,8
2	5,02	51	20,3
3	5,04	49	19,4
4	4,98	45	18,1
5	5,02	42	16,7
6	4,97	42	16,9
7	4,97	51	20,5
8	5,03	54	21,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	48,2	Particles
Standard deviation	4,58	Particles
χ^2 (CHI-Quadrat)	3,05	
Probability	**88**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	19,3	mg/kg
Standard deviation	1,83	mg/kg
rel. Standard deviaton	9,5	%
Horwitz standard deviation	10,2	%
HorRat-value	**0,93**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA ptAL07 Spiking Level Sample

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	46	18,3
2	4,99	46	18,4
3	5,02	40	15,9
4	5,00	39	15,6
5	5,01	42	16,8
6	4,98	51	20,5
7	4,98	46	18,5
8	4,97	47	18,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	44,6	Particles
Standard deviation	4,10	Particles
χ^2 (CHI-Quadrat)	2,64	
Probability	**92**	%
Recovery rate	92	%

Normal distribution		
Number of samples	8	
Mean	17,9	mg/kg
Standard deviation	1,64	mg/kg
rel. Standard deviaton	9,2	%
Horwitz standard deviation	10,4	%
HorRat-value	**0,89**	
Recovery rate	92	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL07 - 2020***
PT name	***Allergens VII: Crustaceae, Peanut and Coconut in Instant Product with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *Asian instant noodle soup (ground) / Ingredients: Noodles 89% (wheat flour, potato starch, palm oil, salt, acidity regulators: E501, E500, E339, antioxidant E306, emulsifier E322, seasoning), spice powder 9% (hydrolyzed vegetable protein, maltodextrin, yeast extract, salt, wheat flour, black pepper, garlic, corn flour), vegetable-mushroom flakes 2% (pak choi, shitake, textured vegetable protein, carrots, red chili peppers, onions), 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: Crustaceae (Crustaceae protein, DNA), Peanut (Peanut protein, DNA), Coconut (Coconut 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 January 08th 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, Ph.D.*

** 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
		Deutschland/Germany
		USA
		ITALIEN/ITALY
		KANADA/CANADA
		ITALIEN/ITALY
		Deutschland/Germany
		SCHWEDEN/SWEDEN
		Deutschland/Germany
		KANDA/CANADA
		Deutschland/Germany
		Deutschland/Germany
		SCHWEIZ/SWITZERLAND
		Deutschland/Germany
		GROSSBRITANNIEN/GREAT BRITAIN
		ITALIEN/ITALY
		GROSSBRITANNIEN/GREAT BRITAIN
		GROSSBRITANNIEN/GREAT BRITAIN
		USA
		Deutschland/Germany
		USA
		VIETNAM
		GROSSBRITANNIEN/GREAT BRITAIN

[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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