Proficiency Tests
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Evaluation Report
proficiency test

DLA 07/2017

Allergens VII:

Peanut and Mollusks

in Soup Powder

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

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Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

Two PT-samples with the same food matrix were provided for the detection and quantitative determination of the allergens in the range of mg/kg as well as one spiking level sample with a simple matrix. One of the samples (spiked sample) and the spiking level sample contain the respective allergenic ingredients in a similar concentration range. The results of the spiking level sample should give the possibility of a comparison with the spiked sample in respect to the detectability of the allergens with and without the influence of matrix and / or food processing. *Due to the relatively low content of mullusks two additional samples sample M and spiking level M were provided.*

The test material is a common in commerce tomato cream soup (powder) with addition of potato flour. The basic composition of samples A, B and M was the same (see table 1a + 1b). After sieving (mesh 2,5 mm) the basic mixture was homogenized.

Afterwards the **spiked samples A and M** were produced as follows:
The spiking materials (premix) containing the allergenic ingredients peanut and squid were sieved by means of a centrifugal mill (sample A mesh 250 µm, sample M mesh 500 µm) 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 homogenization.

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

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

Table 1a: Composition of DLA-Samples A, B and Spiking Level Sample

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Tomato Cream Soup (Powder) Ingredients: Tomato powder (39%), sugar, starch, wheat flour, iodised salt, seasoning, yeast extract, palm oil, onions, garlic, acidifier: citric acid, flavor, thickener: guar gum, celery seed, herbs Nutrients per 100 g: Protein 10 g, Carbohydrates 65 g, Fat 2 g	89,7 g/100g	90,0 g/100g	-
Potato Flour	9,7 g/100g	9,7 g/100g	
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	< 0,3 g/100g	-	99,9 g/100 g
Peanuts, roasted: milled, mixture (18 products from USA, Asia, Africa, South America) - as Peanut* - *thereof 23,2% total protein***	 14,4 mg/kg 3,3 mg/kg	-	 18,4 mg/kg 4,3 mg/kg
Squid (Illex argentinus): dried (60°C), milled - as Squid powder* - *thereof 34% total protein***	 9,9 mg/kg 3,4 mg/kg	-	 12,6 mg/kg 4,3 mg/kg
further Ingredients: Maltodextrin, sodium chloride, sodium sulfate and silicon dioxide	<0,1 g/100 g	-	<0,1 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,46 for peanut protein and general factor F=6,25 for squid protein)

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

Table 1b: Composition of DLA-Sample M and Spiking Level Sample M

Ingredients	**Sample M**	**Spiking Level Sample M**
Tomato Cream Soup (Powder) Ingredients: Tomato powder (39%), sugar, starch, wheat flour, iodised salt, seasoning, yeast extract, palm oil, onions, garlic, acidifier: citric acid, flavor, thickener: guar gum, celery seed, herbs Nutrients per 100 g: Protein 10 g, Carbohydrates 65 g, Fat 2 g	89,7 g/100g	-
Potato Flour	9,7 g/100g	
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	99,7 g/100 g
Peanuts, roasted: milled, mixture (18 products from USA, Asia, Africa, South America) - as Peanut* - *thereof 23,2% total protein***	-	-
Squid (Illex argentinus): dried (60°C), milled - as Squid powder* - *thereof 34% total protein***	 79,1 mg/kg 26,9 mg/kg	 69,8 mg/kg 23,7 mg/kg
further Ingredients: Maltodextrin, sodium chloride, sodium sulfate and silicon dioxide	<0,3 g/100 g	<0,3 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,46 for peanut protein and general factor F=6,25 for squid protein)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT sample A and the spiking level sample and sample M and spiking level M showed a probability of 40%, 46%, 98% and 98%, 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 values of 1,5, 1,3, 0,6 and 0,7, respectively. The HorRat value of 1,5 for sample A is slightly increased, but homogeneity tests by ELISA proved sufficient homogeneity of sample A (s. below). The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample A by all ELISA tests for peanut (Immunolab, Veratox and AgraQuant) (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.6 and 3.8) [3].

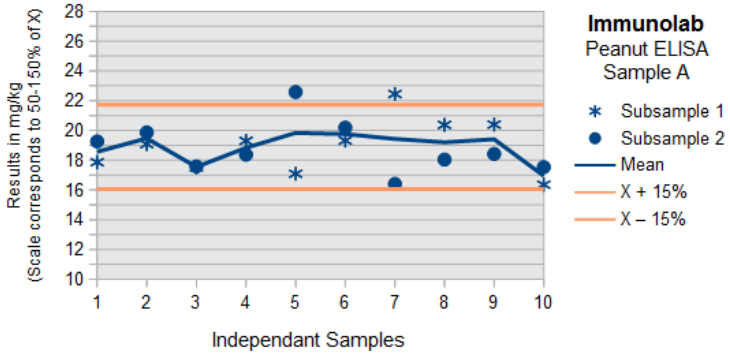
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Erdnuss ELISA

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

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	17,9	19,3	18,6
2	19,1	19,9	19,5
3	17,5	17,6	17,5
4	19,3	18,4	18,8
5	17,1	22,6	19,8
6	19,3	20,2	19,8
7	22,4	16,4	19,4
8	20,4	18,0	19,2
9	20,4	18,4	19,4
10	16,4	17,5	16,9

General average X	18,9	
SD of sample means Sx	0,96	5,1%
SD within-samples Sw	1,43	7,6%
SD between-samples Ss	< 0,96	< 5,1%

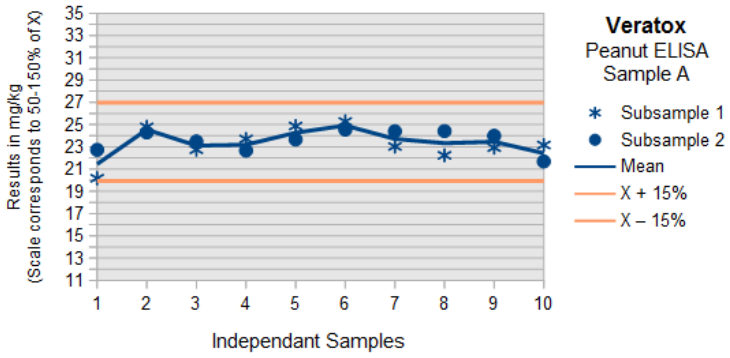


Neogen Veratox ELISA Peanut

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Peanut 23,4 ± 0,9 mg/kg

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

General average X	23,4	
SD of sample means Sx	1,02	4,3%
SD within-samples Sw	0,71	3,0%
SD between-samples Ss	0,88	3,8%

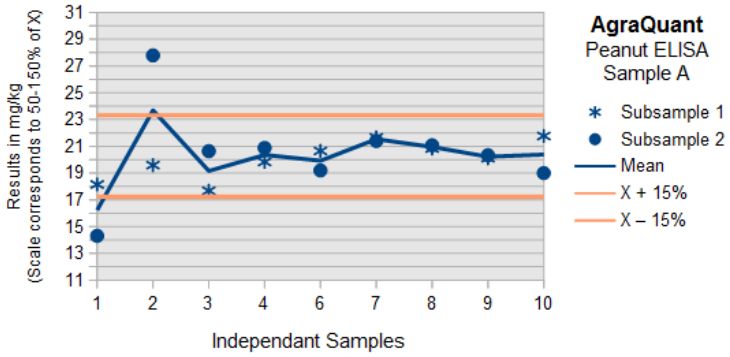


Romerlabs AgraQuant Peanut

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Erdnuss 20,3 ± 1,6 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	18,1	14,3	16,2
2	19,6	27,8	23,7
3	17,7	20,6	19,2
4	19,8	20,9	20,4
6	20,7	19,2	19,9
7	21,7	21,4	21,5
8	20,8	21,1	21,0
9	20,1	20,3	20,2
10	21,8	19,0	20,4

General average X	20,3	
SD of sample means Sx	1,98	9,8%
SD within-samples Sw	1,68	8,3%
SD between-samples Ss	1,59	7,8%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,35 (23,3°C) The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A, B and the spiking level sample were sent to every participating laboratory in the 45th week of 2017 and sample M and spiking level sample M in the 50th week of 2017. The testing method was optional. The tests should be finished at December 22nd 2017 and January 26th 2018, respectively.

With the cover letter along with the sample shipment the following information was given to participants:

a) (1. Letter)
There are two different samples A and B possibly containing the allergenic parameters peanut (roasted) and/or molluscs (squid, dried) in the range of mg/kg in the matrix of soup powder. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing.

b) (Information by email and 2. letter)
Due to the relatively low level of molluscs (here squid) in the previously shipped PT samples, we have decided to send an additional sample M and an additional spiking level sample M.
As announced by email find enclosed the additional sample M and an additional spiking level sample M. These samples contain higher levels of molluscs (squid, dried) and can be analysed optionally (peanut is not contained!).

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

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

All 14 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 valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

If possible, this is the standard procedure for the evaluation of ELISA methods for the determination of allergens:

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

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

respectively) [3].

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

i) ***Robust standard deviation of all results*** - S^x_{ALL}
ii) ***Robust standard deviation of single methods*** - $S^x_{METHOD\ i}$ with at least 5 quantitative results given.

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

The relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) given in table 3a (ELISA) and table 3b (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 11 – 32% for the ELISA methods and 24 – 42% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

The Working Group on Prolamin Analysis and Toxicity (WGPAT) coordinated a collaborative study with two commercial ELISA test kits for the determination of gluten using the monoclonal R5 antibody [24]. 12 food samples with gliadin in the range of 0 - 168 mg/kg were analyzed by 20 laboratories. Recovery rates ranged between 65 and 110%, relative repeatability deviations ranged from 13 - 25% (method 1) and 11 - 22% (method 2) while the relative reproducibility standard deviations ranged from 23 - 47% (method 1) and 25 - 33% (method 2). According to the authors both ELISA test kits fulfilled therefore the current validation criteria for ELISA methods [24].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [27]. The mean values for two matrices were in the concentration range of 0,3 - 16,1 mg/kg and 1,2 - 20,4 mg/kg, respectively. The lowest relative reproducibility standard deviations of the five test kits were for dark chocolate in the range of 20 - 42% and for cookies in the range of 23 – 61%.

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-34]

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

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and 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) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty of the assigned value

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 ($U_{(x_{pt})}$) for this PT is calculated as follows [3]:

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

If $U_{(x_{pt})} \leq 0{,}3\ \sigma_{pt}$ 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 Quotient $U(x_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

3.9 Figures

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

3.10 Recovery rates: Spiking

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

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done separately for ELISA and PCR-techniques. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

The following result sections are structured equally for the allergenic components. First all results of ELISA or PCR methods for a certain parameter are reported for samples A and B (qualitative / possibly quantitative) and afterwards for the spiking level sample (quantitative). The recovery rates of results for the spiking level sample and the spiked sample A or B are reported then.

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

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

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

For **squid / mollusks** there were only a few quantitative results. For estimation of the recovery rates the ELISA results given as mollusk protein (Elution Technologies) were converted by DLA to total food items **(squid, dried)** using the analysed protein content of the raw material (see page 5). The results given as **squid, fresh** (Immunolab) were converted by DLA to **dried squid** using a water content of 80% in fresh squid (USDA Nutrient Data Base and Souci/Fachmann/Kraut Nutrition Tables).

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		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data:		
Target standard deviation σ_{pt}		
lower limit of target range (X_{pt} - 2σ_{pt})		
upper limit of target range (X_{pt} + 2σ_{pt})		
Quotient S/σ_{pt}*		
Standard uncertainty $U_{(Xpt)}$		
Quotient $U_{(Xpt)}/\sigma_{pt}$		
Number of results in target range		
Percent in target range		

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

4.1 Proficiency Test Peanut

4.1.1 ELISA Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
13	positive	20,3	negative	<1.0	2/2 (100%)	AQ	
7	positive	18,0	negative	< 1	2/2 (100%)	BK	
14	positive	20,0	negative	<1	2/2 (100%)	BK	
12b	positive	24,0	negative	<1	2/2 (100%)	BK	
6	positive	123	negative	< 4	2/2 (100%)	IL	result converted °
8	positive	18,6	negative	< 0.1	2/2 (100%)	IL	
10a	positive	17,3	negative	<0,3	2/2 (100%)	NL	
1	positive	42,8	negative	< 0,13	2/2 (100%)	RS-F	
3	positive	25,0	negative	<1.5	2/2 (100%)	RS-F	
4	positive	24,5	negative	<2.5	2/2 (100%)	RS-F	
5	positive	30,9	negative	< 2,5	2/2 (100%)	RS-F	
9	positive	140	negative		2/2 (100%)	RS-F	result converted °
11	positive	25,1	negative	<2.5	2/2 (100%)	RS-F	
10b	positive	17,7	negative	<0,13	2/2 (100%)	RS-F	
12a	positive	23,7	negative	<2,5	2/2 (100%)	VT	

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	Sample A		Sample B	
Number positive	15		0	
Number negative	0		15	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
13	20,3	-0,42		AQ	
7	18,0	-0,82		BK	
14	20,0	-0,47		BK	
12b	24,0	0,23		BK	
6	123	18		IL	result converted ° / and excluded
8	18,6	-0,72		IL	
10a	17,3	-0,94		NL	
1	42,8	3,5	6,8	RS-F	
3	25,0	0,41	-0,01	RS-F	
4	24,5	0,32	-0,09	RS-F	
5	30,9	1,5	0,93	RS-F	
9	140	21	18	RS-F	result converted ° / and excluded
11	25,1	0,43	0,01	RS-F	
10b	17,7	-0,9		RS-F	
12a	23,7	0,18		VT	

° calculation S. 20

Methods:
AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

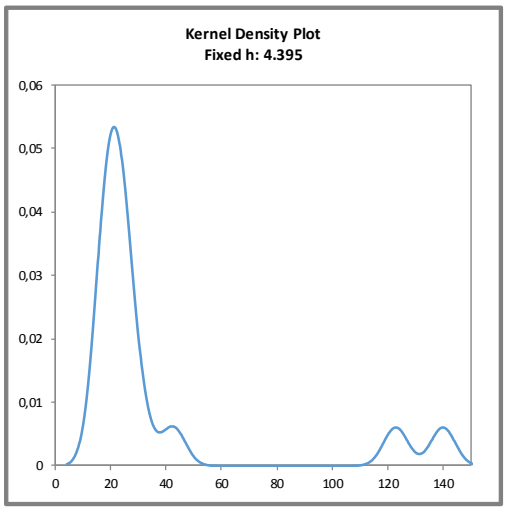

Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a side-peak caused by one result at 43 mg/kg (method RS-F) and two peaks > 100 mg/kg due to two outliers (possibly caused by mistake giving results as peanut protein).

Characteristics: Quantitative evaluation ELISA: Peanut

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
*Number of results ***	13	6
Number of outliers	0	0
Mean	23,7	27,7
Median (only *RS-F: X_{pt}*)	23,7	**25,1**
Robust Mean (X_{pt})	**22,7**	27,2
Robust standard deviation (S*)	**5,05**	**8,50**
Target range:		
Target standard deviation σ_{pt}	**5,67**	**6,27**
lower limit of target range	**11,3**	**12,5**
upper limit of target range	**34,0**	**38,6**
Quotient S/σ_{pt}*	*0,89*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*1,75*	*4,30*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,31*	*0,69*
Results in the target range	*12*	*5*
Percent in the target range	*92*	*83*

*** without evaluation no. 6 and no. 9*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results. The results with evaluation numbers 6 and 9 were excluded from statistical calculations.

The evaluation of all methods and the evaluation of results from method RS-F showed a low to normal variability of results. For valuation of method RS-F the median was used as assigned value (vgl. 3.1). The quotients S/σ_{pt} were 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 assigned values of the evaluation of all results and method RS-F were 158% and 174% of the spiking level of peanut to sample A and thus above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Peanut" p.32).

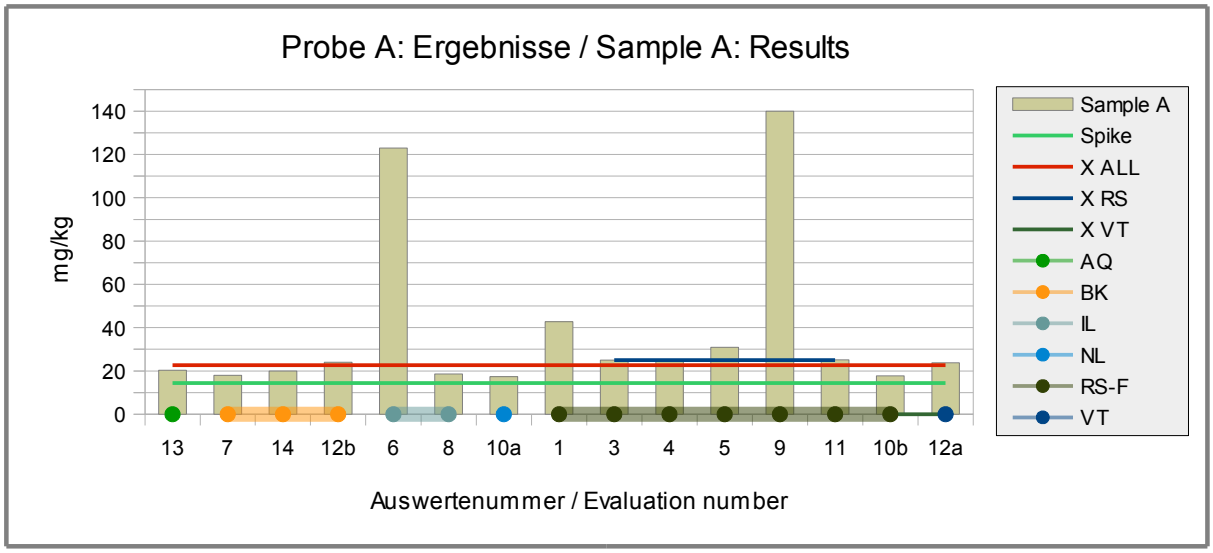

Abb./Fig. 2: ELISA Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

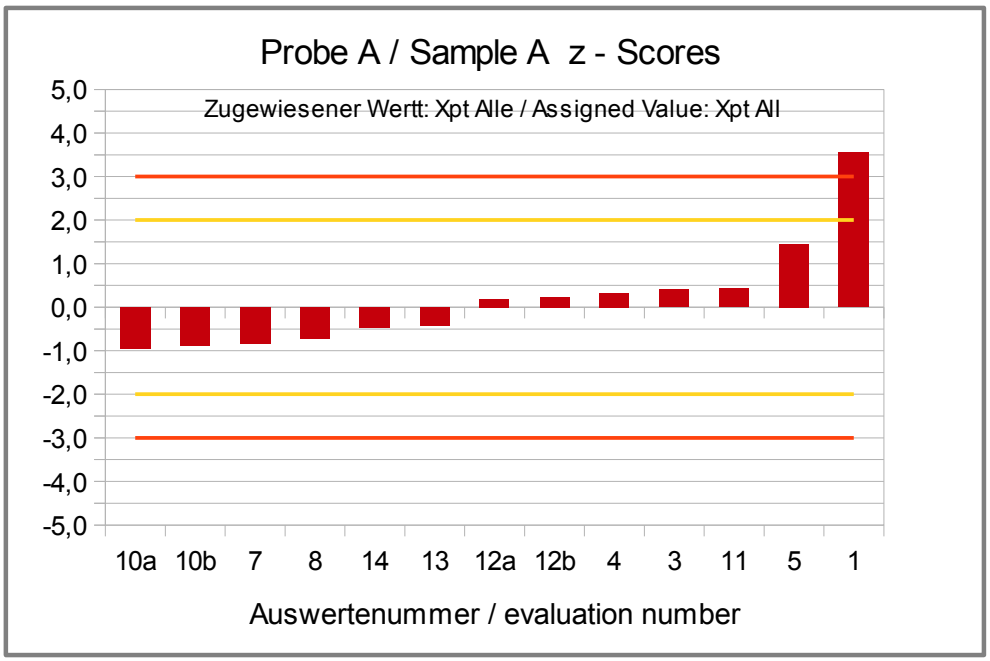

Abb./Fig. 3:
z-Scores (ELISA Results Peanut)
Assigned value robust mean (algorithm A) of all results

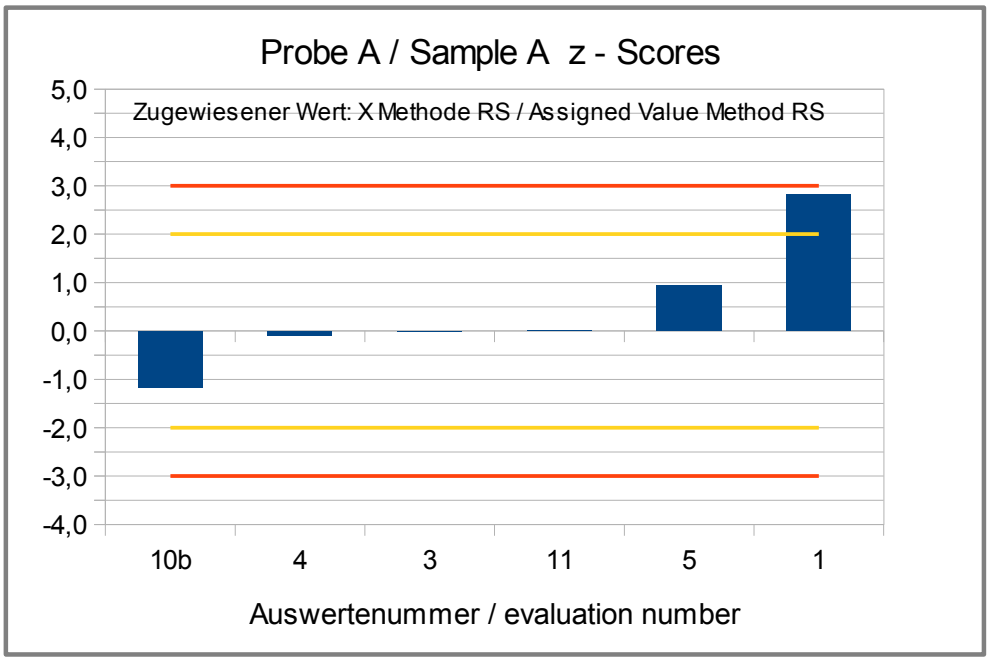

Abb./Fig. 4:
z-Scores (ELISA Results Peanut)
Assigned value median of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
13	>40			AQ	
7	43,0	-0,45		BK	
14	48,5	0,00		BK	
12b				BK	
6	188	12		IL	result converted ° / and excluded
8	30,1	-1,5		IL	
10a	27,8	-1,7		NL	
1	100	4,3	6,8	RS-F	
3	60,0	0,95	0,10	RS-F	
4	56,7	0,68	-0,13	RS-F	
5	60,3	1,0	0,12	RS-F	
9	248	16	13	RS-F	result converted ° / and excluded
11	51,5	0,25	-0,48	RS-F	
10b	31,6	-1,4		RS-F	
12a	51,2	0,22		VT	

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Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

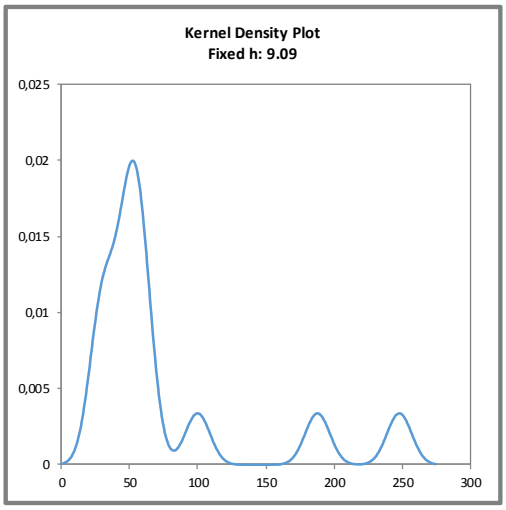

Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a slight shoulder and a side-peak caused by one result at approx. 100 mg/kg (method RS-F) and two additional peaks > 150 mg/kg due to two outliers (possibly caused by mistake giving results as peanut protein).

Characteristics: Quantitative evaluation 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 ***	11	6
Number of outliers	0	0
Mean	51,0	60,0
Median	51,2	58,4
Robust Mean (X)	**48,5**	**58,6**
Robust standard deviation (S*)	**16,10**	**21,9**
Target range:		
Target standard deviation σ_{pt}	**12,1**	**14,60**
lower limit of target range	**24,2**	**29,3**
upper limit of target range	**72,7**	**87,8**
Quotient S/σ_{pt}*	*1,3*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*6,08*	*11,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,50*	*0,76*
Results in the target range	*10*	*5*
Percent in the target range	*91*	*83*

*** without evaluation no. 6 and no. 9*

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 results with evaluation numbers 6 and 9 were excluded from statistical calculations.

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

The assigned values of the evaluation of all results and method RS-F were 264% and 318% of the spiking level of peanut to the spiking level sample and thus clearly above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Peanut" p.32).

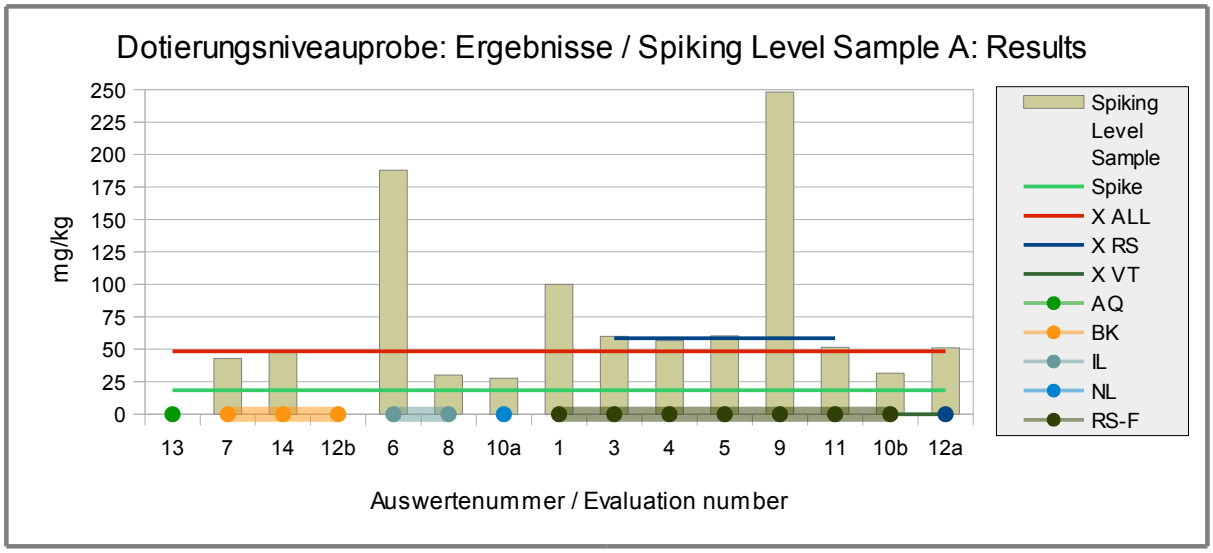

Abb./Fig. 6: ELISA Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

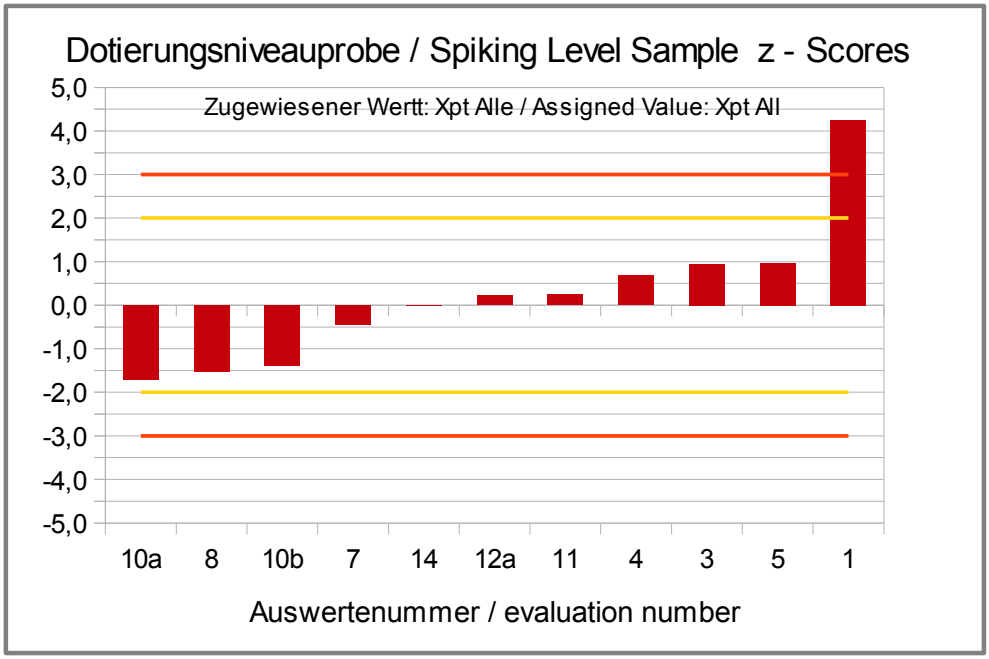

Abb./Fig. 7:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

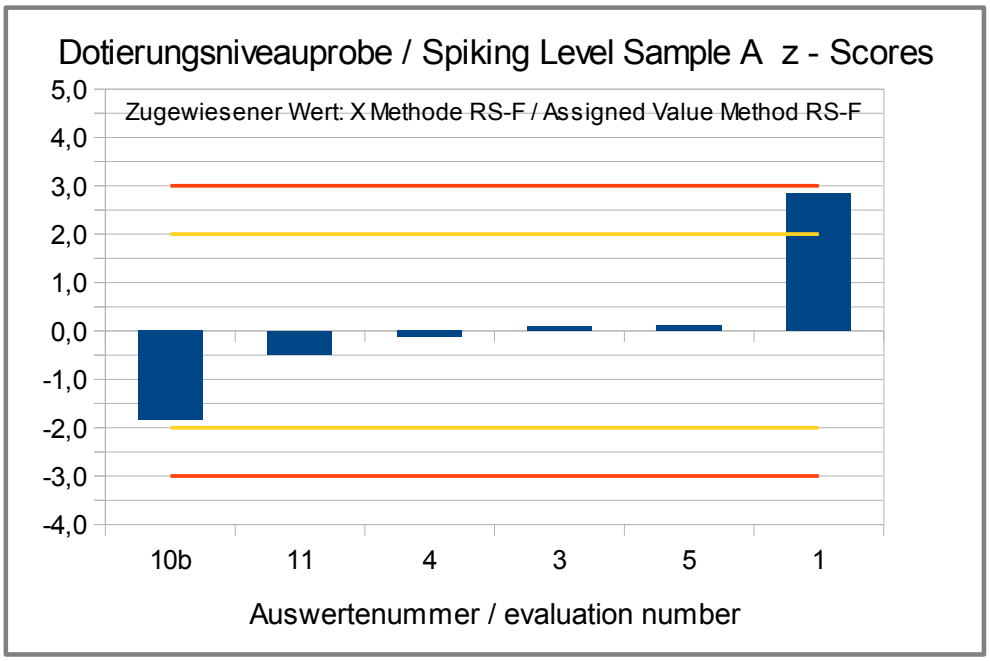

Abb./Fig. 8:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates ELISA for Peanut:
Spiking level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
13	>40		20,3	**141**	AQ	
7	43,0	234	18,0	**125**	BK	
14	48,5	264	20,0	**139**	BK	
12b			24,0	167	BK	
6	188	1024	123	857	IL	result converted °
8	30,1	164	18,6	**130**	IL	
10a	27,8	151	17,3	**121**	NL	
1	100	545	42,8	298	RS-F	
3	60,0	327	25,0	174	RS-F	
4	56,7	309	24,5	171	RS-F	
5	60,3	328	30,9	215	RS-F	
9	248	1351	140	976	RS-F	result converted °
11	51,5	280	25,1	175	RS-F	
10b	31,6	172	17,7	**123**	RS-F	
12a	51,2	279	23,7	165	VT	

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RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**6**
Percent in RA	**0**	Percent in RA	**40**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking level sample by ELISA methods none of the recovery rates were within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 40% (6) of the recovery rates were within the range of acceptance.

4.1.2 PCR Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
7	positive		negative		2/2 (100%)	ASU	
2	positive		negative		2/2 (100%)	MS	
1	positive	> 4	negative	< 1	2/2 (100%)	SFA-ID	
3	positive	> 1	negative	< 1	2/2 (100%)	SFA-ID	
11	positive	7,31	negative	< 1	2/2 (100%)	SFA-ID	
9	positive	< BG	negative		2/2 (100%)	SFA-Q	
13	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	7		0	
Number negative	0		7	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

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

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

Quantitative Valuation PCR: Sample A

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

(Quantitative) Valuation PCR: Spiking Level Sample

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

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positive			ASU	
2	positive			MS	
1	positive	> 4		SFA-ID	
3	positive	> 1		SFA-ID	
11	positive	21,1		SFA-ID	
9	positive	< BG		SFA-Q	
13	positive			div	

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

Methoden:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = not indicated / other method

Comments:

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

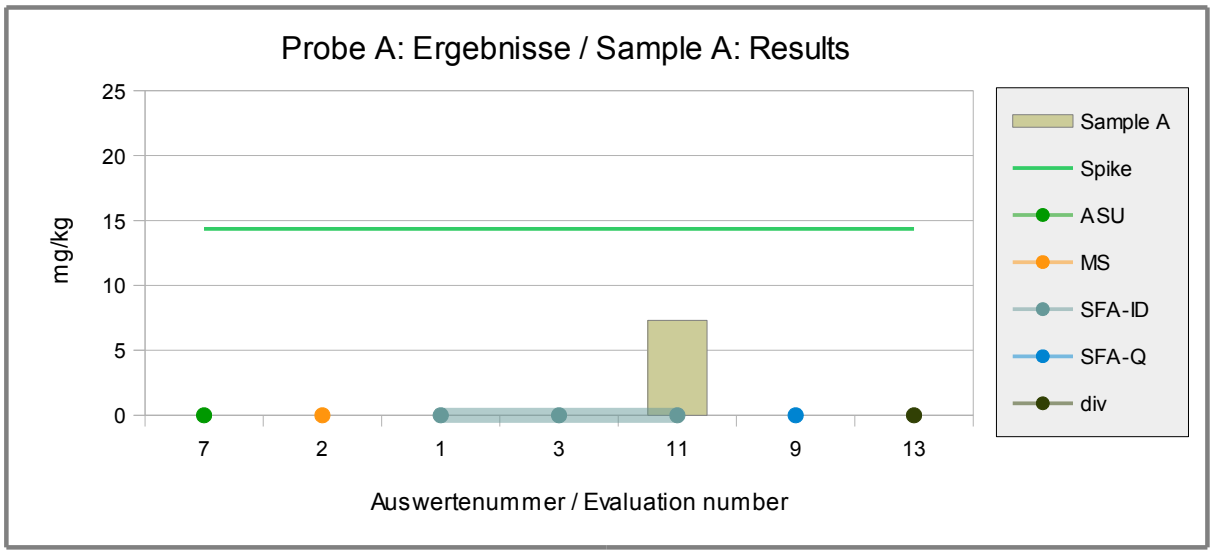

Abb./Fig. 9: PCR Results Peanut Sample A
green line = Spiking level
round symbols = Applied methods (see legend)

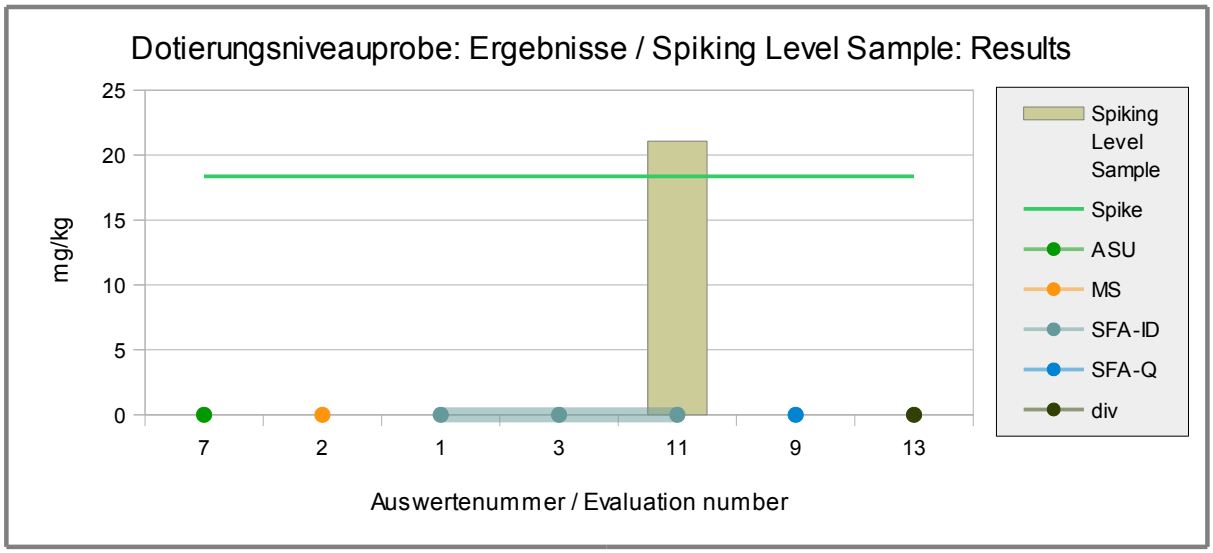

Abb./Fig. 10: PCR Results Peanut Spiking Level Sample
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Peanut:
Spiking Material Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
7					ASU	
2					MS	
1	> 4		> 4		SFA-ID	
3	> 1		> 1		SFA-ID	
11	21,1	**115**	7,31	**51**	SFA-ID	
9	< BG		< BG		SFA-Q	
13					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	100	Percent in RA	100

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

** Range of acceptance of AOAC for allergen ELISAS

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

Comments:
One participant obtained recovery rates by PCR for the spiking material sample and for the spiked food matrix sample A both within the range of the AOAC-recommendation of 50-150% for.

4.2 Proficiency Test Mollusks

4.2.1 ELISA Results: Mollusks (Squid, dried)

Qualitative valuation of results: Samples A, B and M

Evaluation number	Sample A	Sample A	Sample B	Sample B	Sample M	Sample M	Qualitative Valuation*	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with spiking		
11					positive	1,86	1/1 (100%)	ET	as Mollusk protein
12	negative	<1	negative	< 1	positive	2,20	2/3 (67%)	ET	als Mollusk protein
8	positive	2,5	negative	< 1,7	positive	10,0	3/3 (100%)	IL	as Squid, fresh

Methods:
ET = Elution Technologies ELISA Kit
IL = Immunolab

Comments:
By ELISA methods 3 participants detected mollusks (squid) in sample M and one participant in sample A.
** The qualitative valuation of results was done by comparison to the spiking of the samples (s. pages 5-6).*

Quantitative Valuation ELISA: Samples A and M

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

Qualitative valuation of results: Spiking Level Sample and Spiking Level Sample M

Evaluation number	Spiking Level Sample		Spiking Level Sample M		Qualitative Valuation*	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with spiking		
11			positive	3,08	1/1 (100%)	ET	as Mollusk protein
12	negative	<1	positive	14,0	1/2 (50%)	ET	als Mollusk protein
8	positive	3,50	positive	25,0	2/2 (100%)	IL	as Squid, fresh

Methods:
ET = Elution Technologies ELISA Kit
IL = Immunolab

Comments:
By ELISA methods 3 participants detected mollusks (squid) in spiking level sample M and one participant in the spiking level sample.
** The qualitative valuation of results was done by comparison to the spiking of the samples (s. pages 5-6).*

Quantitative Valuation ELISA: Spiking Level Samples

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

Recovery Rates ELISA for Mollusks (Squid, dried): Spiking level Sample M and Sample M

Evaluation number	Spiking Level Sample M	Recovery rate*	Sample M	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
11	9,06	13	5,47	7	ET	result converted °
12	41,2	**59**	6,47	8	ET	result converted °
8	5,00	7	2,00	3	IL	result converted °

° calculation S. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	0
Percent in RA	33	Percent in RA	0

Methods:
ET = Elution Technologies ELISA Kit
IL = Immunolab

* Recovery rate 100% relative size: Squid, dried, s. page 6

** Range of acceptance of AOAC for allergen ELISAS

Comments:
One participant obtained a recovery rate by ELISA for the spiking material sample M within the range of the AOAC-recommendation of 50-150% for. All other recovery rates were below the range of acceptance.

4.2.2 PCR Results: Mollusks (Squid, dried)

Qualitative valuation of results: Samples A, B and M

Evaluation number	Sample A	Sample A	Sample B	Sample B	Sample M	Sample M	Qualitative Valuation*	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with spiking		
3	negative	< 0,4	negative	< 0,4	positive	>0.4	2/3 (67%)	SFA-ID	
9	negative		negative		positive		2/3 (67%)	SFA-ID	
11	-		-		negative	< 1	0/1 (0%)	SFA-ID	no positive sample detected
13	negative		negative		negative		1/3 (33%)	SFA-ID	no positive sample detected
14	positive		negative		-		2/2 (100%)	SFA-ID	
10	positive		negative		positive		3/3 (100%)	div	

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

Comments:
By PCR methods 2 of 5 participants detected mollusks (squid) in sample A and 3 of 5 participants in sample M.
** The qualitative valuation of results was done by comparison to the spiking of the samples (s. pages 5-6).*

Quantitative Valuation PCR: Samples A and M

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

Qualitative valuation of results: Spiking Level Samples and Spiking Level Sample M

Evaluation number	Spiking Level Sample		Spiking Level Sample M		Qualitative Valuation*	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with spiking		
3	negative	< 0,4	positive	> 0,4	1/2 (50%)	SFA-ID	
9	negative		positive		1/2 (50%)	SFA-ID	
11			negative	<1	0/1 (0%)	SFA-ID	no positive sample detected
13	negative		negative		0/2 (0%)	SFA-ID	no positive sample detected
14	positive		-		1/1 (100%)	SFA-ID	
10	positive		positive		2/2 (100%)	div	

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

Comments:
By PCR methods 2 of 5 participants detected mollusks (squid) in the spiking level sample and 3 of 5 participants in spiking level sample M.
** The qualitative valuation of results was done by comparison to the spiking of the samples (s. pages 5-6).*

Quantitative Valuation PCR: Spiking Level Samples

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

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 ELISA: Peanut

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
AQ	13	26.01.18	positive	20,27	negative	<1.0	positive	>40	Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
BK	7	20/21.11	positive	18	negative	< 1	positive	43	Peanut	BioKits Peanut Assay Kit, Neogen
BK	14	17.11./22.11.17	positive	20	negative	<1	positive	48,5	Peanut	BioKits Peanut Assay Kit, Neogen
BK	12b	19.12.17	positive	24	negative	<1	positive	n/a	Peanut	BioKits Peanut Assay Kit, Neogen
IL	6	27.11.17	-	28,6	-	<1,0	-	43,5	Peanutprotein	Immunolab Peanut ELISA
IL	8	14.11.17	positive	18,6	negative	< 0.1	positive	30,1	Peanut	Immunolab Peanut ELISA
NL	10a	04.12.17	positive	17,33	negative	<0,3	positive	27,81	Peanut	nutriLinia Peanut-E ELISA (NC-6014), RomerLabs
RS-F	1	20/12	positive	42,78	negative	< 0,13	positive	100,07	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	3	21.12.17	positive	25	negative	<1.5	positive	60	Peanut	R6202 Ridascreen Fast Peanut
RS-F	4		24,5	PPM	<2.5	PPM	56,7	PPM	Please select!	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	5	19.12.17	positive	30,9	negative	< 2,5	positive	60,3	Peanut	Ridascreen Fast Peanut (R6202), R-Biopharm
RS-F	9	14.11.17	positive	32,5P	negative		positive	57,5P	Peanutprotein	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	11	09.01.18	positive	25,12	negative	<2.5	positive	51,48	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	10b	04.12.17	positive	17,7	negative	<0,13	positive	31,58	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
VT	12a	04.12.17	positive	23,7	negative	<2,5	positive	51,2	Peanut	Veratox Peanut, Neogen

***Continuation ELISA Peanut*:**

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	Meth. Abk.
AQ	13		ELISA kit instructions follow ed	Yes	
BK	7	Polyclonal AB against Conarachin (Ara h1)	As Per Kit Instructions	yes	
BK	14				
BK	12b			yes	
IL	6			no	
IL	8	Peanutprotein			
NL	10a		As Per Kit Instructions	yes	
RS-F	1	The antibodies specifically detect peanut proteins, including the peanut allergen Ara h 1 and Ara h 2	According to kit manua	yes	
RS-F	3				
RS-F	4				
RS-F	5		As Per Kit Instructions, w ith Skimmed Milk Pow der	yes	
RS-F	9		cross-reactive to lentils, green peas, fenugreek	yes	LOD 0,8 mg/kg, LOQ 2,4 mg/kg, Article no. R6202
RS-F	11	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	10b		As Per Kit Instructions	no	Method in training period
VT	12a			yes	

5.1.2 ELISA: Mollusks

Samples A, B and Spiking Level Sample

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
ET	12	07.12.17	negative	<1	negative	<1	-		Mollusk protein	Elution Technologies ELISA Kit Mollusk Protein E-75MSK
IL	8	14.11.17	positive	2,5	negative	< 1.7	positive	3,5	Squid, fresh	Immunolab Mollusc ELISA

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ET	12			yes	
IL	8	Mollusc Tropomyosin			

Sample M and Spiking Level Sample M

Meth. Abr.	Evaluation number	Date of analysis	Result Sample M		Result Spiking Sample M		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
ET	11	15.01.18	positive	1,86	positive	3,08	Mollusk protein	Elution Technologies ELISA Kit Mollusk Protein E-75MSK
ET	12	29.12.17	positive	2,2	positive	14	Mollusk protein	Elution Technologies ELISA Kit Mollusk Protein E-75MSK
IL	8	18.12.17	positive	10	positive	25	Squid, fresh	Immunolab Mollusc ELISA

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ET	11	As Per Kit Instructions	As Per Kit Instructions	No	
ET	12			yes	
IL	8	Mollusc Tropomyosin			

5.1.3 PCR: Peanut

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
ASU	7	16.11.17	positive		negative		positive		Peanut	ASU L44.00-11 vom Januar 2013
MS	2	14.11.	positive		negative		positive		Peanut-DNA	Microsynth
SFA-ID	1	19/12	positive	> 4	negative	< 1	positive	> 4	Peanut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	3	21.12.17	positive	>1	negative	<1	positive	>1	Peanut-DNA	SureFood® ALLERGEN Peanut Art.-No. S3103 Congen
SFA-ID	11	06.01.18	positive	7,31	negative	<1	positive	21,08	Peanut	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	9	15.11.17	positive	<LOQ	negative		positive	<LOQ	Peanut-DNA	Sure Food Allergen Quant, R-Biopharm / Congen
div	13		positive		negative		positive		Peanut-DNA	other: please fill in!

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	7	86bp sequence of gene of Ara h2	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
MS	2		Macherey Nagel Nucleo Spin Food optimized: increased sample weight, buffer change (washing step with Lysis Buffer) Rnase step, Chloroform step, 2xCQW; RealTime PCR with 45 cycles, Decontamination step with UNG; Inhibition control	yes/no	
SFA-ID	1	Peanut-DNA	According to kit manua	yes	
SFA-ID	3				
SFA-ID	11	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA-Q	9			yes	LOD 1 mg/kg, LOQ 4 mg/kg, Artikelnr. S3203
div	13	ITS	Tris extraction wirth column clean up, end-point PCR.	No	in-house

5.1.4 PCR: Mollusks

Samples A, B and Spiking Level Sample

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
SFA-ID	3	21.12.17	negative	<0.4	negative	<0.4	negative	<0.4	Mollusk-DNA	SureFood® ALLERGEN Molluscs Art.-No. S3113 Congen
SFA-ID	9	15.11.17	negative		negative		negative		Mollusk-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13		negative		negative		negative		Mollusk-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	14	08.12.17	positive		negative		positive			Sure Food Allergen ID, R-Biopharm / Congen
div	10		positive		negative		positive		Squid-DNA	Espineira et al 2010: Species authentication of octopus, bobtail and bottle squids (families *Octopodidae, Sepiidae* and *Sepiolidae*) by FINS methodology in seafoods.

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-ID	3				
SFA-ID	9			yes	LOD 0,4 mg/kg, Article no. S3113
SFA-ID	13	Unknown	Tris extraction w ith magnetic bead clean up	Yes	DNA yield >200ng/ul but no amplification seen.
SFA-ID	14				
div	10	cytB, 652 bp	DNA clean-up w ith Promega Wizard, additional Amylase digest, conventional PCR follow ed by sequencing	yes	Sequencing of positive samples proved *Ilex argentinus*

Continuation PCR Mollusks:

Sample M and Spiking Level Sample M

Meth. Abr.	Evaluation number	Date of analysis	Result Sample M		Result Spiking Sample M		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
SFA-ID	3		Pos	>0.4	Pos	>0.4	Mollusk-DNA	SureFood® ALLERGEN Molluscs Art.-No. S3113 Congen
SFA-ID	9	05.01.18	positive		positive		Mollusken-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	11	21.12.17	negative	<1	negative	<1	Mollusk, fresh	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13	26.01.18	negative		negative		Please select!	Sure Food Allergen ID, R-Biopharm / Congen
div	10		positive		positive		Squid-DNA	Espineira et al 2010: Species authentication of octopus, bobtail and bottle squids (families *Octopodidae, Sepiidae* and *Sepiolidae*) by FINS methodology in seafoods.

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-ID	3			yes	
SFA-ID	9			yes	LOD 0,4 mg/kg, Article no. S3113
SFA-ID	11	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA-ID	13		Bead beater DNA extraction with magentic bead clean up, RTPCR with PCR kit		Samples had >200ng/ul DNA extracted but little/no amplification seen.
div	10	cytB, 652 bp	DNA clean-up with Promega Wizard, additional Amylase digest, conventional PCR followed by sequencing	yes	Sequencing of positive samples proved *Ilex argentinus*

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 07-2017 Sample A

Weight whole sample	2,72	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	18,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	40	15,9
2	5,13	50	19,5
3	5,07	34	13,4
4	5,00	35	14,0
5	4,96	52	21,0
6	5,03	39	15,5
7	5,02	39	15,5
8	5,00	44	17,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	41,6	Particles
Standard deviation	6,59	Particles
χ^2 (CHI-Quadrat)	7,31	
Probability	**40**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	16,6	mg/kg
Standard deviation	2,62	mg/kg
rel. Standard deviaton	15,8	%
Horwitz standard deviation	10,5	%
HorRat-value	**1,5**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA 07-2017 Spiking Level Sample

Weight whole sample	1,49	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	66	26,3
2	5,05	57	22,6
3	5,07	73	28,8
4	5,06	72	28,5
5	4,96	54	21,8
6	4,99	53	21,2
7	4,99	55	22,0
8	4,96	60	24,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	61,2	Particles
Standard deviation	7,65	Particles
χ^2 (CHI-Quadrat)	6,69	
Probability	**46**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	24,4	mg/kg
Standard deviation	3,05	mg/kg
rel. Standard deviaton	12,5	%
Horwitz standard deviation	9,89	%
HorRat-value	**1,3**	
Recovery rate	113	%

Microtracer Homogeneity Test
DLA 07-2017 Sample M

Weight whole sample	0,54	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	36,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	3,32	68	41,0
2	3,36	69	41,1
3	3,45	74	42,9
4	3,50	67	38,3
5	3,50	75	42,9
6	3,60	78	43,3
7	3,55	68	38,3
8	3,26	62	38,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	70,1	Particles
Standard deviation	3,86	Particles
χ^2 (CHI-Quadrat)	1,49	
Probability	**98**	%
Recovery rate	111	%

Normal distribution		
Number of samples	8	
Mean	40,7	mg/kg
Standard deviation	2,24	mg/kg
rel. Standard deviaton	5,51	%
Horwitz standard deviation	9,16	%
HorRat-value	**0,60**	
Recovery rate	111	%

Microtracer Homogeneity Test
DLA 07-2017 Spiking Level Sample M

Weight whole sample	0,34	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	41,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	3,38	69	40,8
2	3,04	67	44,1
3	2,80	56	40,0
4	2,55	55	43,1
5	3,20	66	41,3
6	3,23	70	43,3
7	3,15	73	46,3
8	2,75	65	47,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,2	Particles
Standard deviation	3,90	Particles
χ^2 (CHI-Quadrat)	1,63	
Probability	**98**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	43,3	mg/kg
Standard deviation	2,59	mg/kg
rel. Standard deviaton	5,98	%
Horwitz standard deviation	9,07	%
HorRat-value	**0,66**	
Recovery rate	104	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 07-2017***
PT name	***Allergens VII:*** **Peanut and Molluscs in Soup Powder**
Sample matrix (processing)	***Samples A + B:*** *Tomato Cream Soup (Powder)/ ingredients: tomato powder, sugar, wheat flour, salt, seasoning, maltodextrin, yeast extract, palm fat, onion, garlic, acidifier: citric acid, thickener: guar gum, celery / potato powder, other food additives and allergenic foods peanut roasted and squid dried (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods (peanut roasted and squid dried)*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: cooled 2 - 10°C (long term < -18°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *peanut (peanut protein, DNA), molluscs (squid powder) (molluscs protein, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest December 22nd 2017***
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, PhD*

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

2nd letter - Information on the proficiency test (PT):

PT number	***DLA 07-2017***
PT name	***Allergens VII:*** **Peanut and Molluscs in Soup Powder**
Sample matrix (processing)	***Sample M:*** *Tomato Cream Soup (Powder)/ ingredients: tomato powder, sugar, wheat flour, salt, seasoning, maltodextrin, yeast extract, palm fat, onion, garlic, acidifier: citric acid, thickener: guar gum, celery / potato powder, other food additives and allergenic food squid dried (one of both samples)* ***Spiking Level Sample M:*** *potato powder, other food additives and allergenic food (squid dried)*
Number of samples and sample amount	*1 Sample M: 25 g* *+ 1 Spiking Level Sample M: 15 g*
Storage	*Sample M: cooled 2 - 10°C (long term < -18°C)* *Spiking Level Sample M: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *molluscs (squid powder) (molluscs protein, DNA)* *Sample M: < 500 mg/kg* *Spiking Level Sample M: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest January 26th 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler, PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country

[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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