Proficiency Tests
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Evaluation Report
proficiency test

DLA 05/2016

Allergens V:

Peanut and Almond

in Pastry (Butter Cookies)

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

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Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.

Erratum:
Seite 6 mittlere Überschrift muss lauten „Homogenität der abgefüllten dotierten Probe B“
Page 6 middle headline must be „Homogeneity of bottled spiked sample B“
(corrected by M.Besler 02/02/2017)

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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 for the detection of allergens in the range of mg/kg and one spiking material sample were provided for analysis. The spiking material sample contains the respective allergenic ingredients in the range of 1-10 % and was added to the spiked PT-sample. The results of the spiking material sample should give the possibility of a comparison with the spiked sample in respect to the detectability of the allergens with and without the influence of matrix and / or food processing.

The test material are a common in commerce butter cookies. The basic composition of both sample A and sample B was the same (see table 1). After crushing, sieving and homogenization of the basic mixture a baked cookie (190°C, 20 min) with added spiking material containing the allergenic ingredients peanut and almond was added to sample B.
The procedure was as follows: After crushing and homogenization the baked cookie containing the allergenic ingredients was added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 5 additional steps and mechanically homogenized in each case until the total quantity had been reached.
After pre-crushing the basic mixture was prepared by means of a centrifugal mill (mesh 1,5 mm) prior to use.

The composition of the spiking material sample and the amounts of allergens in sample B is given in table 2.

After homogenization the samples were portioned to approximately 25 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Butter Cookies Ingredients: Wheat flour, sugar, butter, glucose syrup, baking agents: ammonium hydrogencarbonate and sodium hydrogencarbonate, salt, whole egg powder, acid regulator: sodium carbonate Nutrients per 100 g: Protein 7,5 g, Carbohydrates 75 g, Fat 12 g	100 g/100g	96,3 g/100g
Cookies (baked 190°C, 20 min) Ingredients: sugar, wheat flour, butter, eggs, salt	-	3,6 g/100g
Spiking material sample	-	0,12 g/100g

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Amounts in Sample B**
Potato flour	88,00%	0,12%
Peanut mush Ingredients: Peanuts hot air roasted (99,2%), salt - as Peanut* - *thereof 30% total protein***	8450 mg/kg (= 0,85 %) 8370 mg/kg 2510 mg/kg	 10 mg/kg 2,9 mg/kg
Almond mush, white Ingredients: White almonds - as Almond* - *thereof 20% total protein***	12900 mg/kg (= 1,29 %) 12900 mg/kg 2580 mg/kg	 15 mg/kg 3,0 mg/kg
additional ingredients: *other nuts, legumes and fructose*	< 10,0 %	< 0,02 %

*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)
*** Protein contents according to label

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 10-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 showed a probability of 90% for the spiked sample B and of 30% for the spiking material sample. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a Hor-Rat value of 0,8 and 1,5 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 13528:2009 Annex B.

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample B by all ELISA tests for peanut (Immunolab, AgraQuant Plus and Veratox) and almond (Immunolab, AgraQuant Plus and Veratox), respectively (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [16, 17, 20, 21].

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].

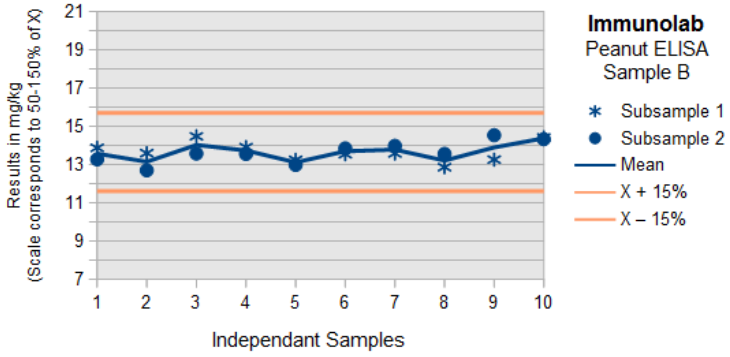
ELISA-Tests: Erdnuss / Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 13,7 ± 0,38 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	13,87	13,27	13,57
2	13,60	12,70	13,15
3	14,47	13,57	14,02
4	13,91	13,55	13,73
5	13,25	12,98	13,11
6	13,54	13,84	13,69
7	13,58	13,98	13,78
8	12,86	13,56	13,21
9	13,26	14,53	13,89
10	14,42	14,33	14,37

General average X	13,65	
SD of sample means Sx	0,41	3,0%
SD within-samples Sw	0,48	3,5%
SD between-samples Ss	0,38	2,8%

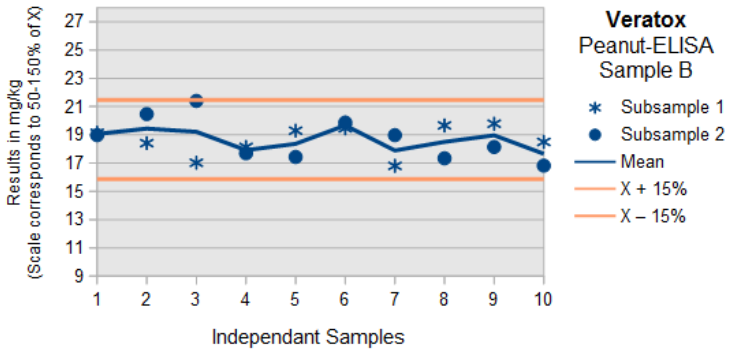


Neogen Veratox ELISA Peanut

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Peanut 18,7 ± 1,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	19,14	18,97	19,06
2	18,41	20,47	19,44
3	17,02	21,40	19,21
4	18,14	17,69	17,92
5	19,30	17,43	18,36
6	19,46	19,87	19,66
7	16,80	18,97	17,89
8	19,67	17,34	18,50
9	19,79	18,14	18,96
10	18,50	16,82	17,66

General average X	18,67	
SD of sample means Sx	0,70	3,8%
SD within-samples Sw	1,46	7,8%
SD between-samples Ss	1,38	7,4%

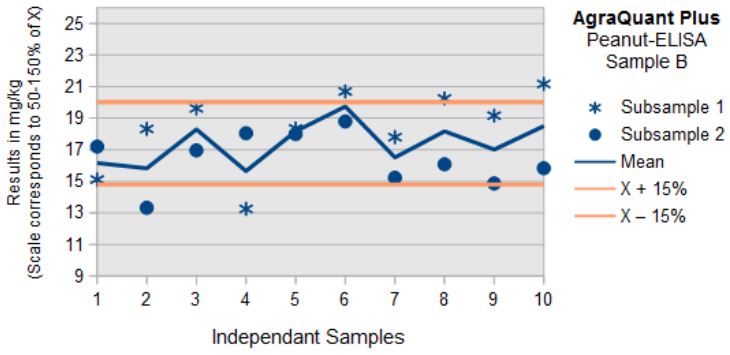


Romerlabs AgraQuant Plus Peanut

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

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	15,12	17,20	16,16
2	18,32	13,33	15,83
3	19,62	16,96	18,29
4	13,26	18,05	15,65
5	18,35	17,99	18,17
6	20,69	18,79	19,74
7	17,80	15,23	16,51
8	20,25	16,08	18,17
9	19,16	14,87	17,02
10	21,17	15,83	18,50

General average X	17,40	
SD of sample means Sx	1,36	7,8%
SD within-samples Sw	2,59	14,9%
SD between-samples Ss	2,40	13,8%



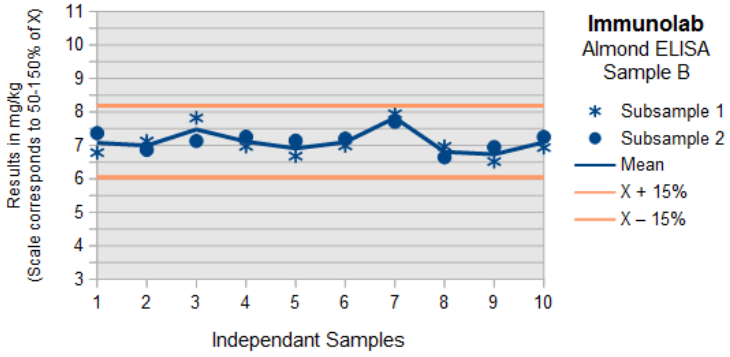
ELISA-Tests: Mandel / Almond

Immunolab Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 7,11 ± 0,18 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,78	7,37	7,08
2	7,13	6,87	7,00
3	7,82	7,13	7,48
4	6,97	7,26	7,11
5	6,68	7,14	6,91
6	6,99	7,20	7,10
7	7,95	7,70	7,82
8	6,97	6,64	6,81
9	6,52	6,95	6,73
10	6,93	7,25	7,09

General average X	7,11	
SD of sample means Sx	0,32	4,5%
SD within-samples Sw	0,29	4,1%
SD between-samples Ss	0,18	2,5%

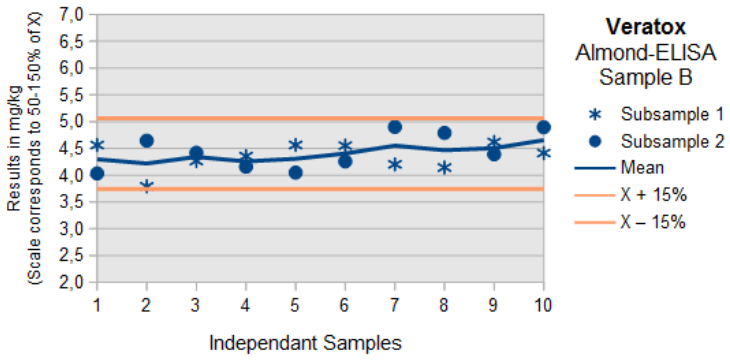


Neogen Veratox ELISA Almond

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Almond 4,40 ± 0,35 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	4,56	4,03	4,30
2	3,79	4,64	4,22
3	4,26	4,42	4,34
4	4,36	4,16	4,26
5	4,56	4,05	4,31
6	4,55	4,26	4,40
7	4,20	4,90	4,55
8	4,14	4,79	4,47
9	4,62	4,39	4,50
10	4,41	4,89	4,65

General average X	4,40	
SD of sample means Sx	0,14	3,2%
SD within-samples Sw	0,36	8,2%
SD between-samples Ss	0,35	7,9%

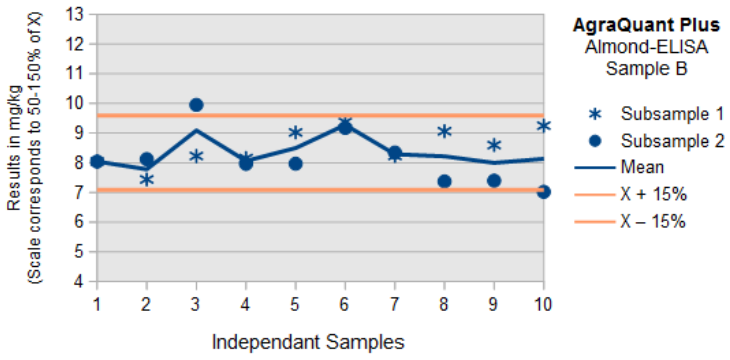


Romerlabs AgraQuant Plus Almond

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 8,34 ± 0,76 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	8,04	8,04	8,04
2	7,44	8,12	7,78
3	8,23	9,95	9,09
4	8,16	7,96	8,06
5	9,02	7,97	8,50
6	9,38	9,17	9,28
7	8,23	8,35	8,29
8	9,06	7,37	8,22
9	8,60	7,40	8,00
10	9,25	7,02	8,14

General average X	8,34	
SD of sample means Sx	0,48	5,8%
SD within-samples Sw	0,83	10,0%
SD between-samples Ss	0,76	9,1%



2.2 Sample shipment and information to the test

The portions of test material (sample A and sample B as well as the spiking material sample) were sent to every participating laboratory in the 35th week of 2016. The testing method was optional. The tests should be finished at October 14th 2016 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 of baked cookies possibly containing the allergenic ingredients peanut and/or almond in the range of mg/kg. Additionally a "Spiking Material Sample" is provided which was used for the spiking of the positive sample (A or B). It contains 1-10% of the allergenic items in potato flour and should be analysed like a normal sample (eventually diluted).
In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Every suitable method for detection or determination of the analytes may be applied (e.g. ELISA, PCR).

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.
During evaluation DLA eventually requests detailed information by email on the type of indicated quantitative results from participants concerned.
Queried and documented were the indicated results and details of the test methods like specifity, 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.
One participant submitted no results. All other 20 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 [23, 24, 25, 26]. 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.

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].
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) ***Robust mean of all results*** - $X_{pt_{ALL}}$
ii) ***Robust mean 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]. 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 identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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-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 3a: 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} [27-28]

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 – 33% for the ELISA methods and 15 – 43% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 3a and 3b).

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 [22]. 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 [22].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [25]. 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 3b: 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} [29-30]

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 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 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 [20], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [17-19], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [21] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [16].

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 [16-22]	**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 [16]	**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 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 3.6.1.

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 material 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 2. 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 [21]. For quantitative PCR 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 analyte are reported for sample A and afterwards for sample B. The results of the spiking material sample are reported together with the referring spiked sample in the recovery section.

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.
In the present PT all ELISA results were uniformly expressed as peanut or almond, therefore no conversions were made.

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 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		
17	negative	<1	positive	10,4	2/2 (100%)	BC	Mean calculated by DLA
3	negative	<1	positive	9,20	2/2 (100%)	BK	
20a	negative	<1.0	positive	11,0	2/2 (100%)	BK	
19	negative	< 0.5	positive	13,0	2/2 (100%)	IL	
9	negative	< 0,3	positive	17,0	2/2 (100%)	NL-E	
20b	negative	<2.5	positive	23,0	2/2 (100%)	RS	
1	-		positive	17,8	1/2 (50%)	RS-F	
2	negative	<2.5	positive	16,3	2/2 (100%)	RS-F	
8	negative	<2,5	positive	12,7	2/2 (100%)	RS-F	
11	negative		positive		2/2 (100%)	RS-F	
12	negative	<0,13	positive	14,2	2/2 (100%)	RS-F	
13	negative	< 2,5	positive	13,8	2/2 (100%)	RS-F	
14	negative	< LOD	positive	14,2	2/2 (100%)	RS-F	Mean calculated by DLA
15	negative	< 2,5	positive	15,4	2/2 (100%)	RS-F	
16	negative		positive	14,8	2/2 (100%)	RS-F	
18	negative		positive	12,1	2/2 (100%)	RS-F	Mean calculated by DLA
7	negative		positive	11,9	2/2 (100%)	VT	
20c	negative	<2.5	positive	9,00	2/2 (100%)	VT	

	Sample A		Sample B	
Number positive	0		18	
Number negative	17		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

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

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

Quantitative valuation of results: Sample B

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
17	10,4	-0,9		BC	Mean calculated by DLA
3	9,20	-1,3		BK	
20a	11,0	-0,8		BK	
19	13,0	-0,2		IL	
9	17,0	1,0		NL-E	
20b	23,0	2,8		RS	
1	17,8	1,2	0,9	RS-F	
2	16,3	0,8	0,5	RS-F	
8	12,7	-0,3	-0,5	RS-F	
11				RS-F	
12	14,2	0,2	-0,1	RS-F	
13	13,8	0,1	-0,2	RS-F	
14	14,2	0,2	-0,1	RS-F	Mean calculated by DLA
15	15,4	0,5	0,2	RS-F	
16	14,8	0,4	0,1	RS-F	
18	12,1	-0,4	-0,7	RS-F	Mean calculated by DLA
7	11,9	-0,5		VT	
20c	9,00	-1,4		VT	

Methods:

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

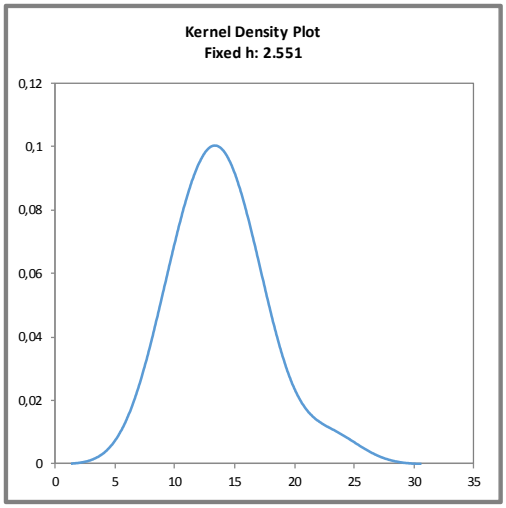


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a normal distribution of results with a slight shoulder at 23 mg/kg (method RS).

Characteristics: Quantitative evaluation Peanut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	17	9
Number of outliers	0	0
Mean	13,9	14,6
Median	13,8	14,2
Robust Mean (X)	**13,6**	**14,6**
Robust standard deviation (S*)	**3,22**	**1,88**
Target range:		
Target standard deviation σ_{pt}	**3,40**	**3,64**
lower limit of target range	**6,80**	**7,28**
upper limit of target range	**20,4**	**21,8**
Quotient S/σ_{pt}*	*0,95*	*0,52*
Standard uncertainty $U_{(Xpt)}$	*0,976*	*0,784*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,29*	*0,22*
Results in the target range	*16*	*9*
Percent in the target range	*94*	*100*

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

Comments to the statistical characteristics and assigned values:

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

The robust means of the evaluation of all results and method RS-F were 136% and 146% of the spiking level of peanut to sample B and within the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Peanut" p.26).

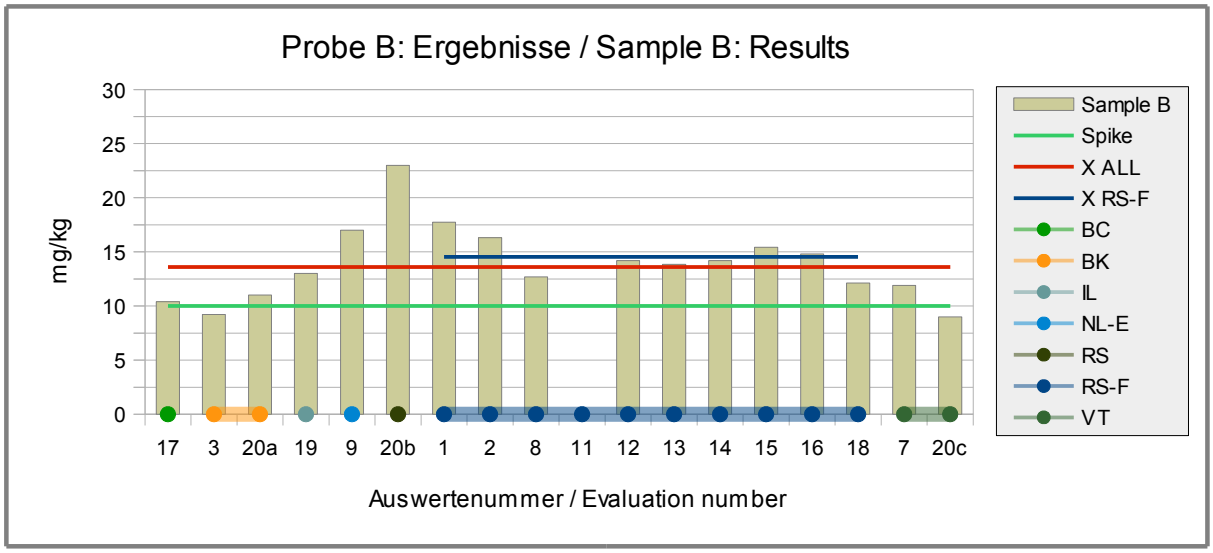


Abb./Fig. 2: ELISA Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

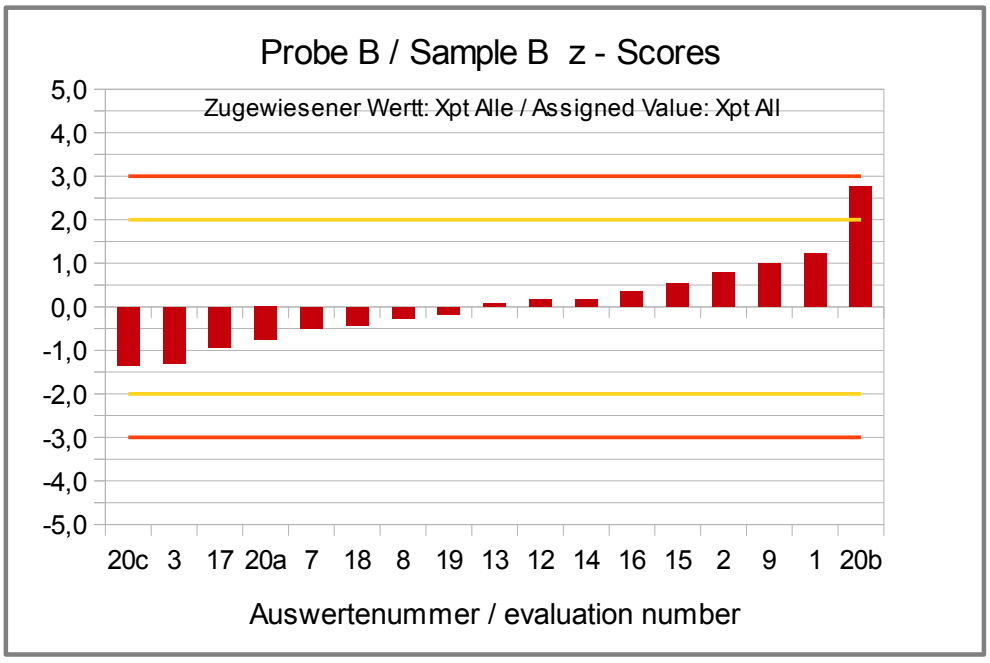


Abb./Fig. 3:
z-Scores (ELISA Results Peanut) Assigned value robust mean of all results

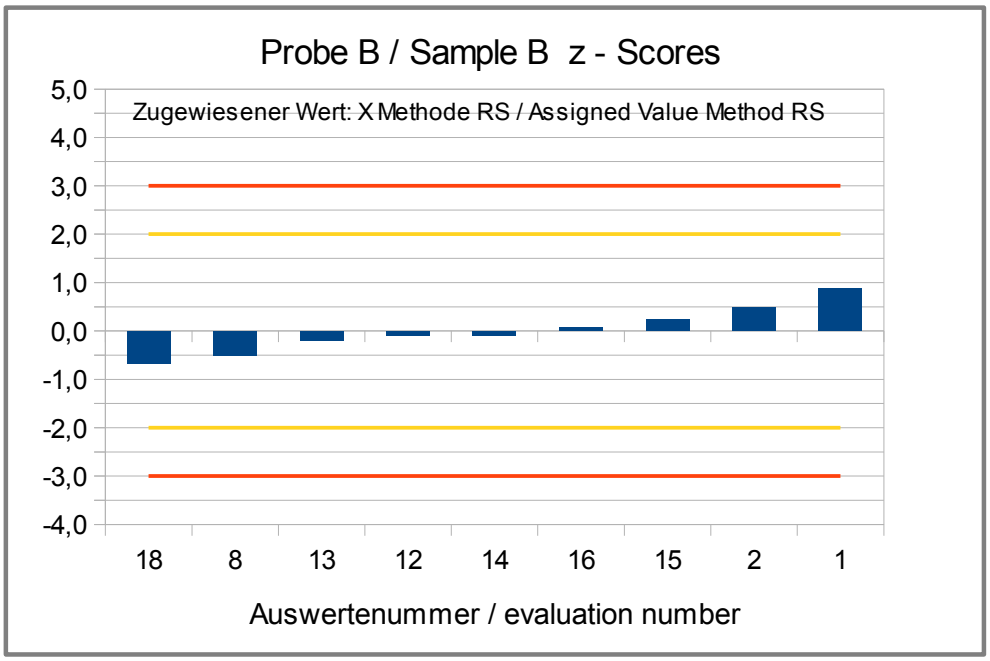


Abb./Fig. 4:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen FAST)

Recovery Rates for Peanut:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
17	23600	282	10,4	**104**	BC	Means calculated by DLA
3	15000	179	9,20	**92**	BK	
20a	>20'000		11,0	**110**	BK	
19	25000	299	13,0	**130**	IL	
9	41000	490	17,0	170	NL-E	
20b	19700	235	23,0	230	RS	
1	28600	342	17,8	178	RS-F	
2	27700	331	16,3	163	RS-F	
8	22700	271	12,7	**127**	RS-F	
11					RS-F	
12	34700	415	14,2	**142**	RS-F	
13			13,8	**138**	RS-F	
14	29800	356	14,2	**142**	RS-F	Means calculated by DLA
15	28400	339	15,4	154	RS-F	
16			14,8	**148**	RS-F	
18	30400	363	12,1	**121**	RS-F	Means calculated by DLA
7	na		11,9	**119**	VT	
20c			9,00	**90**	VT	

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**12**
Percent in RA	**0**	Percent in RA	**71**

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

** Range of acceptance of AOAC for allergen ELISAS

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

Comments:
The recovery rates of the participants ELISA results for the spiking material sample were in the range of 179-490% (mean 325%). The recovery rates (for peanut mush) in PTs of the preceding years were similarly high in the spiking material samples (DLA 06/2015 mean 385% and DLA 05/2014 mean 232%).
For the baked food matrix sample B produced with the spiking material sample 71% of the recovery rates were in the range of the AOAC-recommendation of 50-150%. For the matrix of pastry the recovery rates were similarly fair in the preceding PT DLA 05/2014.

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		
1	negative		positive		2/2 (100%)	ASU	
2	negative	<1	positive	32,8	2/2 (100%)	SFA-ID	
16	negative		positive		2/2 (100%)	SFA-ID	
4a	negative	-	positive	-	2/2 (100%)	SFA-ID	
4b	negative	< 1	positive	< 4	2/2 (100%)	SFA-Q	
5	negative		positive		2/2 (100%)	div	
6	negative		positive		2/2 (100%)	div	
9	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		8	
Number negative	8		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

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

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

Quantitative valuation of results: Sample B

There were < 5 quantitative results, therefore no statistical evaluation was done.

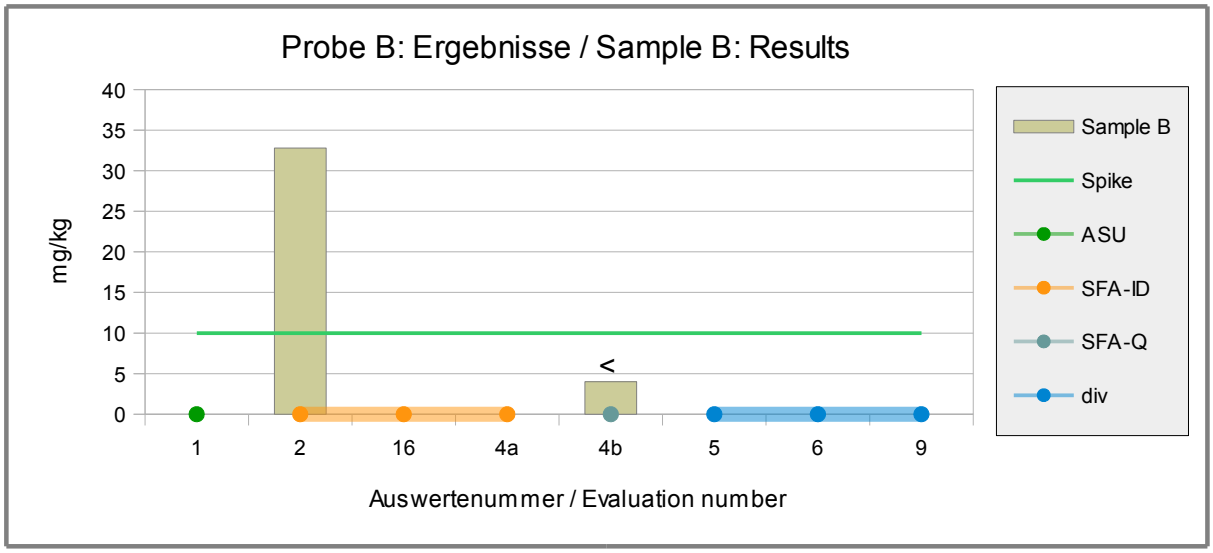


Abb./Fig. 5: PCR Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates for Peanut:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1					ASU	
2	52900	632	32,8	328	SFA-ID	
16					SFA-ID	
4a	-		-		SFA-ID	
4b	5150	**61**	< 4		SFA-Q	
5	83,5	1,0			div	Mean calculated by DLA
6					div	
9					div	

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

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

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
For the spiking material sample one of 3 participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150% by PCR. For the baked food matrix sample B produced with the spiking material sample the recovery rate of the given quantitative result was not in the range of acceptance.

4.2 Proficiency Test Almond

4.2.1 ELISA Results: Almond

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
17	negative	<0.5	positive	6,10	2/2 (100%)	BC	Mean calculated by DLA
19	negative	< 0.2	positive	6,00	2/2 (100%)	IL	
9	negative	< 0,2	positive	4,00	2/2 (100%)	NL-E	
1	-		positive	6,13	1/2 (50%)	RS-F	
2	negative	<2.5	positive	6,08	2/2 (100%)	RS-F	
3	negative	<2,5	positive	8,10	2/2 (100%)	RS-F	
8	negative	<2,5	positive	5,40	2/2 (100%)	RS-F	
10	negative	<2,5	positive	4,50	2/2 (100%)	RS-F	
11	negative		positive		2/2 (100%)	RS-F	
12	negative	<1,2	positive	6,54	2/2 (100%)	RS-F	
13	negative	< 2,5	positive	5,32	2/2 (100%)	RS-F	
14	negative	< LOD	positive	4,51	2/2 (100%)	RS-F	Mean calculated by DLA
15	negative	< 2,5	positive	5,30	2/2 (100%)	RS-F	
16	negative		positive	3,60	2/2 (100%)	RS-F	
18	negative		positive	5,77	2/2 (100%)	RS-F	Mean calculated by DLA
20a	negative	<2.5	positive	6,50	2/2 (100%)	RS-F	
7	negative		positive	5,90	2/2 (100%)	VT	
20b	negative	<2.5	positive	4,00	2/2 (100%)	VT	

	Sample A		Sample B	
Number positive	0		18	
Number negative	17		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

BC = BioCheck ELISA
IL = Immunolab
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of results: Sample B

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
17	6,10	0,5		BC	Mean calculated by DLA
19	6,00	0,4		IL	
9	4,00	-1,1		NL-E	
1	6,13	0,5	0,4	RS-F	
2	6,08	0,4	0,3	RS-F	
3	8,10	1,9	1,8	RS-F	
8	5,40	-0,1	-0,1	RS-F	
10	4,50	-0,7	-0,8	RS-F	
11				RS-F	
12	6,54	0,8	0,7	RS-F	
13	5,32	-0,1	-0,2	RS-F	
14	4,51	-0,7	-0,8	RS-F	Mean calculated by DLA
15	5,30	-0,1	-0,2	RS-F	
16	3,60	-1,4	-1,4	RS-F	
18	5,77	0,2	0,1	RS-F	Mean calculated by DLA
20a	6,50	0,8	0,6	RS-F	
7	5,90	0,3		VT	
20b	4,00	-1,1		VT	

Methoden:
BC = BioCheck ELISA
IL = Immunolab
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

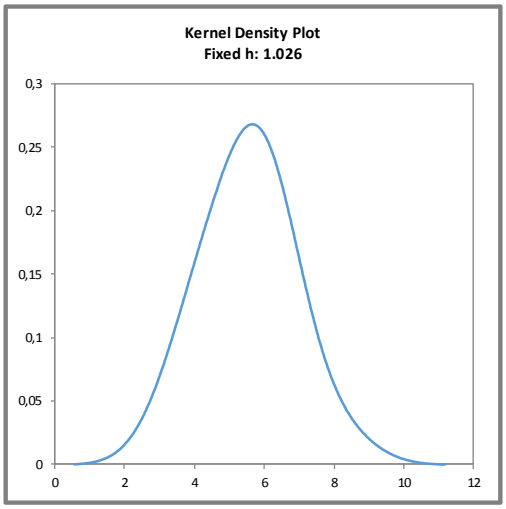


Abb. / Fig. 6:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

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

Characteristics: Quantitative evaluation Almond

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	17	12
Number of outliers	0	0
Mean	5,52	5,65
Median	5,77	5,59
Robust Mean (X)	**5,47**	**5,61**
Robust standard deviation (S*)	**1,13**	**1,09**
Target range:		
Target standard deviation σ_{pt}	**1,37**	**1,40**
lower limit of target range	**2,74**	**2,80**
upper limit of target range	**8,21**	**8,41**
Quotient S/σ_{pt}*	*0,83*	*0,78*
Standard uncertainty $U_{(Xpt)}$	*0,344*	*0,394*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,25*	*0,28*
Results in the target range	*17*	*12*
Percent in the target range	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

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

The robust means of the evaluation of all results and method RS-F were 36% and 37% of the spiking level of almond to sample B and below the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Almond" p.35).

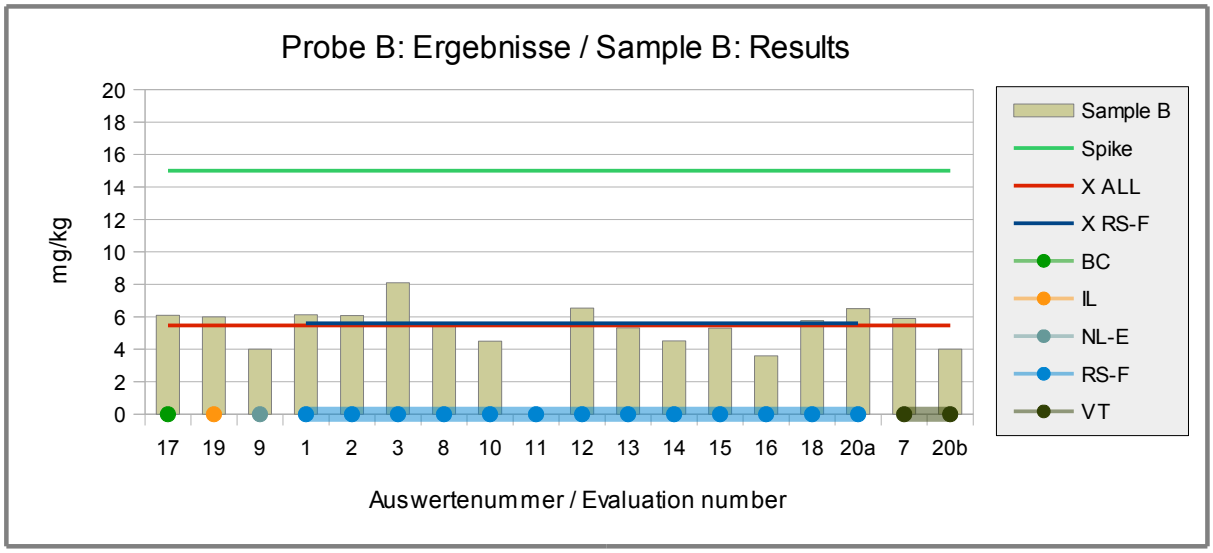


Abb./Fig. 7: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

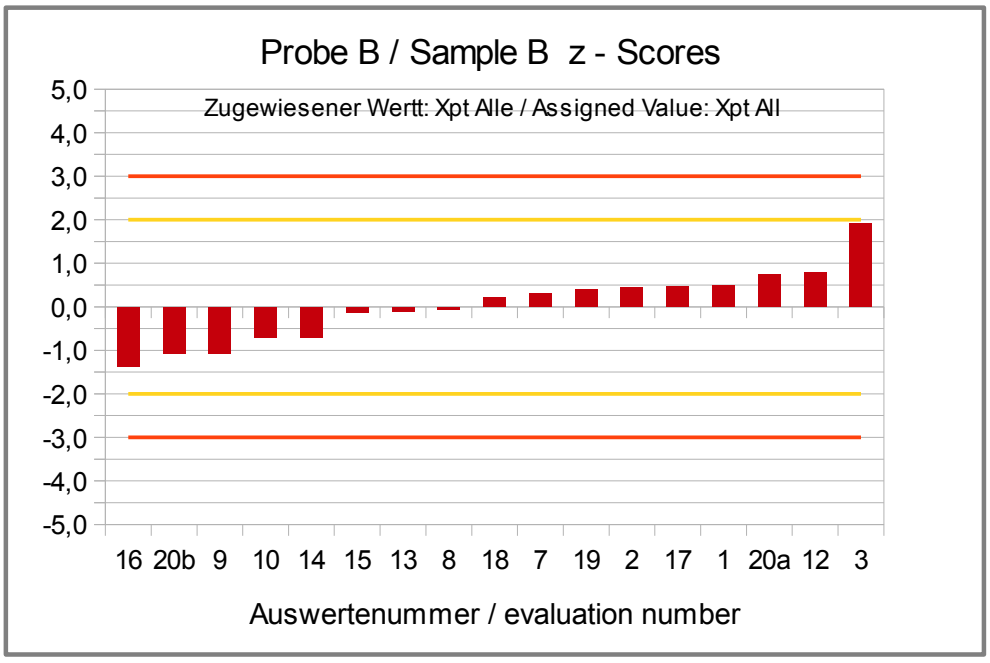


Abb./Fig. 8:
z-Scores (ELISA Results Almond) Assigned value robust mean of all results

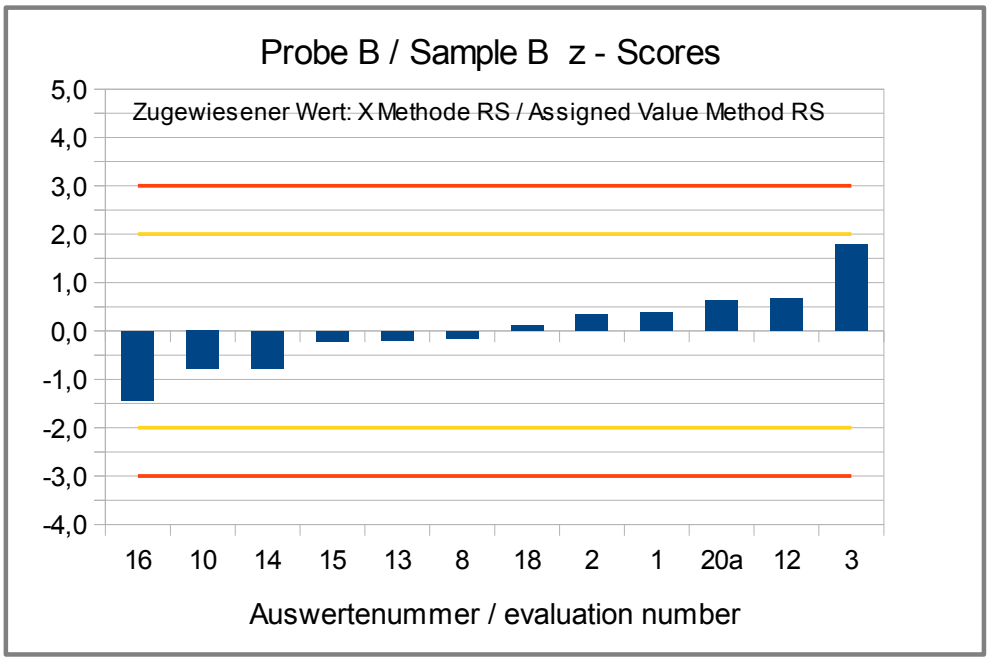


Abb./Fig. 9:
z-Scores (ELISA Results Almond)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen FAST)

Recovery Rates for Almond:
Spiking Material Sample and Sample B

	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
17	11900	**92**	6,10	41	BC	Mean calculated by DLA
19	10000	**78**	6,00	40	IL	
9	10000	**78**	4,00	27	NL-E	
1			6,13	41	RS-F	
2	9550	**74**	6,08	41	RS-F	
3	13000	**101**	8,10	**54**	RS-F	
8	8920	**69**	5,40	36	RS-F	
10	8630	**67**	4,50	30	RS-F	
11					RS-F	
12	10700	**83**	6,54	44	RS-F	
13			5,32	35	RS-F	
14	9480	**73**	4,51	30	RS-F	Mean calculated by DLA
15	11800	**91**	5,30	35	RS-F	
16			3,60	24	RS-F	
18	9970	**77**	5,77	38	RS-F	Mean calculated by DLA
20a			6,50	43	RS-F	
7	na		5,90	39	VT	
20b			4,00	27	VT	

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**1**
Percent in RA	**100**	Percent in RA	**6**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
BC = BioCheck ELISA
IL = Immunolab
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking material sample 100% (11) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the baked food matrix sample B produced with the spiking material sample one of the recovery rates was in the range of acceptance. All other results were between 27-44%.

4.2.2 PCR Results: Almond

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
1	negative		positive		2/2 (100%)	ASU	
2	negative	<1	positive	3,30	2/2 (100%)	SFA-ID	
4	negative	-	negative	-	1/2 (50%)	SFA-ID	
16	negative		positive		2/2 (100%)	SFA-ID	
5	negative		positive		2/2 (100%)	div	
6	negative		positive		2/2 (100%)	div	
9	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		6	
Number negative	7		1	
Percent positive	0		86	
Percent negative	100		14	
Consensus value	negative		positive	

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

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

Quantitative valuation of results: Sample B

There were < 5 quantitative results, therefore no statistical evaluation was done.

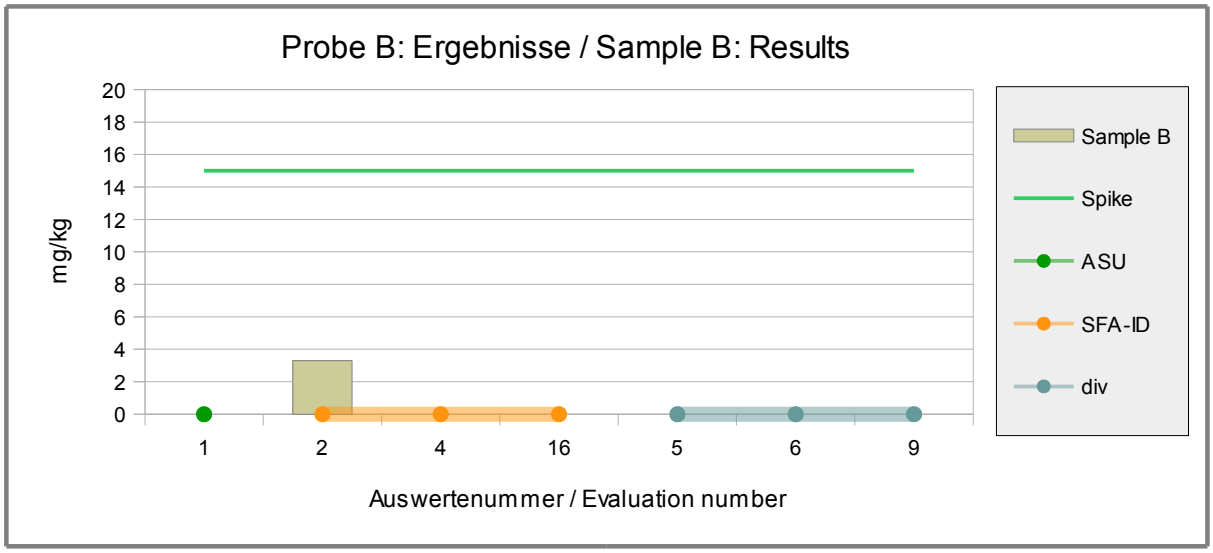


Abb./Fig. 10: PCR Results Almond
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates for Almond:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1					ASU	
2	5010	39	3,30	22	SFA-ID	
4	-		-		SFA-ID	
16					SFA-ID	
5	122	0,95			div	Mean calculated by DLA
6					div	
9					div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

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

Comments:
For the spiking material sample and for the baked food matrix sample B produced with the spiking material sample all recovery rates of the PCR methods were below the range of the AOAC-recommendation of 50-150%.

5. Documentation

5.1 Details by the participants

Note: Information given in German was 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
BC	17	20.09.16	negative	<1	positive	10,2	positive	20800	Peanut	Biocheck peanut Check
BC	17	20.09.16	negative	<1	positive	10,6	positive	26400	Peanut	Biocheck peanut Check
BK	3	06.09.16	negative	<1	positive	9,2	positive	15000	Peanut	BioKits Peanut Assay Kit, Neogen
BK	20a		negative	<1.0	positive	11	positive	>20'000	Peanut	BioKits Peanut Assay Kit, Neogen
IL	19	05.09.16	negative	< 0.5	positive	13	positive	25000	Peanut	Immunolab Peanut ELI-SA
NL-E	9		negative	< 0,3	positive	17	positive	41000	Peanut	nutriLinia Peanut-E ELI-SA (NC-6014), Transia
RS	20b		negative	<2.5	positive	23	positive	19700	Peanut	Ridascreen Peanut (R6201), r-Biopharm
RS-F	1	13.10.16	-		-	17,75	-	28606,17	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	2	05.10.16	negative	<2.5	positive	16,3	positive	27744	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	8		-	<2,5	-	12,7	-	22687	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	11	28.09.	negative		positive		positive		Please select!	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	12		-	<0,13	-	14,19	-	34711	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	13	13.09.16	negative	< 2,50	positive	13,84	-		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	27.09.16	-	< LOD	-	14,91	-	30474	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	27.09.16	-	< LOD	-	13,49	-	29203	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	15	27.09.	negative	< 2,5	positive	15,43	positive	28387,56	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	16		negative		positive	14,82	-		Please select!	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	18	08.09.16	negative		positive	11,19	positive	29200	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	18	08.09.16	negative		positive	13,03	positive	31500	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
VT	7	14.09.16	negative		positive	11,9	na	na	Peanut	Veratox Peanut Allergen, Neogen
VT	20c		negative	<2.5	positive	9	positive		Peanut	Veratox Peanut Allergen, Neogen

continued ELISA Peanut:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
BC	17		60°C extraction	
BC	17		60°C extraction	
BK	3	Conarachin-A	As Per Kit Instructions	
BK	20a			
IL	19			
NL-E	9		As Per Kit Instructions	
RS	20b			
RS-F	1	Ara h1 and Ara h2	As Per Kit Instructions	
RS-F	2	See Kit Instructions	As Per Kit Instructions	
RS-F	8	peanut proteins	Sample B 1:2 diluted, Spiking sample: 1.:4000 diluted	
RS-F	11	See Kit Instructions	As Per Kit Instructions	
RS-F	12			Indication of tw o decimal places not usefull for spiking level *
RS-F	13			
RS-F	14		1 g + 20 ml extraction buffer from kit, 10 min 60°C, centrifugation, dilution according to concetrations	
RS-F	14		1 g + 20 ml extraction buffer from kit, 10 min 60°C, centrifugation, dilution according to concetrations	
RS-F	15		As Per Kit Instructions	
RS-F	16			
RS-F	18			
RS-F	18			
VT	7			
VT	20c			

* DLA recommends at least two decimal places with respect to relevant digits, for example: 2200 mg/kg instead of 2235 mg/kg, and 2,2 mg/kg instead of 2 mg/kg

5.1.2 ELISA: Almond

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Tag/Monat	qualitativ	mg/kg	qualitativ	mg/kg	qualitativ	mg/kg	z.B. Lebensmittel / Protein	Test-Kit + Anbieter
BC	17	20.09.16	negative	<0.5	positive	5,9	positive	13100	Almond	Biocheck Almond Check
BC	17	20.09.16	negative	<0.5	positive	6,3	positive	10600	Almond	Biocheck Almond Check
IL	19	05.09.16	negative	< 0.2	positive	6	positive	10000	Almond	Immunolab Almond ELISA
NL-E	9		negative	< 0,2	positive	4	positive	10000	Almond	nutriLinia Almond-E ELISA (NC-6018), Transia
RS-F	1	12.10.16	-		-	6,13	-		Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	2	05.10.16	negative	<2.5	positive	6,08	positive	9553	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	3	08.09.16	negative	<2,5	positive	8,1	positive	13000	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	8		-	<2,5	-	5,4	-	8919	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	10	07.09.16	negative	<2,5	positive	4,5	positive	8633	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	11	05.10.	negative		positive		positive		Please select!	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	12		-	<1,2	-	6,54	-	10695	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	13	23.09.16	negative	< 2,50	positive	5,32	-		Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	14	27.09.16	-	< LOD	-	4,42	-	9380	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	14	27.09.16	-	< LOD	-	4,59	-	9583	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	15	27.09.	negative	< 2,5	positive	5,3	positive	11762,25	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	16		negative		positive	3,6	-		Please select!	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	18	09.09.16	negative		positive	4,75	positive	10070	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	18	12.09.16	negative		positive	6,79	positive	9870	Almond	Ridascreen Fast Almond (R6901), r-Biopharm
RS-F	20a		negative	<2.5	positive	6,5	positive		Almond	Ridascreen Fast Almond (R6901), r-Biopharm
VT	7	14.09.16	negative		positive	5,9	na	na	Almond	Veratox Almond Allergen, Neogen
VT	20b		negative	<2.5	positive	4	positive		Almond	Veratox Almond Allergen, Neogen

continued ELISA Almond:

Meth. Abr.	Evaluation number	Specifity	rks to the Method (Extraction and Determir	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
BC	17		60°C extraction	
BC	17		60°C extraction	
IL	19			
NL-E	9		As Per Kit Instructions	
RS-F	1		As Per Kit Instructions	
RS-F	2	As Per Kit Instructions	As Per Kit Instructions	
RS-F	3	Almond proteins	As Per Kit Instructions	
RS-F	8	Almond proteins	Spiking sample 1.:2000 diluted	
RS-F	10			Result for Sample B w as reportad as single value since the duplicat renderd a less than 2,5 mk/kg. Result close to our LOQ
RS-F	11	As Per Kit Instructions	As Per Kit Instructions	
RS-F	12			Indication of tw o decimal places not usefull for spiking level
RS-F	13			
RS-F	14		1 g + 20 ml extraction buffer from kit, 10 min 60°C, centrifugation, dilution according to concetrations	
RS-F	14		1 g + 20 ml extraction buffer from kit, 10 min 60°C, centrifugation, dilution according to concetrations	
RS-F	15		As Per Kit Instructions	
RS-F	16			
RS-F	18			
RS-F	18			
RS-F	20a			
VT	7			
VT	20b			

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	1		negative		positive		-		Peanut-DNA	ASU §64 L 44.00-11 (P-CR-Erdnuss)
SFA-ID	2	05.10.16	negative	<1	positive	32,79	positive	52896	Peanut	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	16		negative		positive		-		Please select!	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	4a	09.09.	negative	-	positive	-	positive	-	Peanut	Sure Food Allergen ID, Congen / r-Biopharm
SFA-Q	4b	09.09.	negative	< 1	positive	< 4	positive	5145	Peanut	Sure Food Allergen QUANT, Congen / r-Biopharm
div	5	12.10.16	negative		positive		-	84	Please select!	pmPES
div	5	12.10.16	negative		positive		-	83	Please select!	pmPES
div	6	11.10.16	negative		negative		positive		Please select!	Real-Time PCR (in house method)
div	9		negative		positive		positive		Please select!	in house method

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	1		according to ASU-Method	
SFA-ID	2	See Kit Instructions	As Per Kit Instructions	
SFA-ID	16			
SFA-ID	4a	-	S3103 SureFood® ALLERGEN ID Peanut LOQ 1 mg/kg Extraction by S1053 SureFood® PREP Advanced, Protocol 1	-
SFA-Q	4b	-	S3203 SureFood® ALLERGEN QUANT Peanut LOQ 1 mg/kg, LOD 4 mg/kg Extraction by S1053 SureFood® PREP Advanced, Protocol 1	-
div	5		CTAB, Magnetic Beads	
div	5		CTAB, Magnetic Beads	
div	6			
div	9	Ara h 3	Real Time PCR, 45 Cycles	

5.1.4 PCR: Almond

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	1		negative		positive		-		Almond-DNA	ASU §64 L 18.00-20 (P-CR-Mandel)
SFA-ID	2	24.09.16	negative	<1	positive	3,3	positive	5006	Almond	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	4	14.09.	negative	-	negative	-	positive	-	Almond	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	16		negative		positive		-		Please select!	Sure Food Allergen ID, Congen / r-Biopharm
div	5	12.10.16	negative		positive		-	133	Please select!	pmMad-Hex
div	5	12.10.16	negative		positive		-	111	Please select!	pmMad-Hex
div	6	12.10.16	negative		positive		positive		Please select!	Real-Time PCR (in house method)
div	9		negative		positive		positive		Please select!	in house method

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	1		according to ASU-Method	
SFA-ID	2	See Kit Instructions	As Per Kit Instructions	
SFA-ID	4	-	S3104 SureFood® ALLERGEN ID Almond LOQ 4 mg/kg Extraction by S1053 SureFood® PREP Advanced, Protocol 1	-
SFA-ID	16			
div	5		CTAB, Magnetic Beads	
div	5		CTAB, Magnetic Beads	
div	6			
div	9	nsLTP	Real Time PCR, 45 Cycles	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 05-2016 Sample B

Weight whole sample	2,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,24	55	21,0
2	5,06	58	22,9
3	5,28	57	21,6
4	5,15	59	22,9
5	5,13	59	23,0
6	5,16	53	20,5
7	5,12	54	21,1
8	5,02	44	17,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,9	Partikel
Standard deviation	4,68	Partikel
χ^2 (CHI-Quadrat)	2,79	
Probability	**90**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	21,3	mg/kg
Standard deviation	1,82	mg/kg
rel. Standard deviaton	8,53	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,84**	
Recovery rate	112	%

Microtracer Homogeneity Test
DLA 05-2016 Spiking material sample

Weight whole sample	1,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	48	19,2
2	5,09	55	21,6
3	5,01	40	16,0
4	5,54	43	15,5
5	5,15	61	23,7
6	5,30	60	22,6
7	5,30	48	18,1
8	5,30	50	18,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	50,7	Partikel
Standard deviation	7,79	Partikel
χ^2 (CHI-Quadrat)	8,38	
Probability	**30**	%
Recovery rate	85	%

Normal distribution		
Number of samples	8	
Mean	19,5	mg/kg
Standard deviation	2,99	mg/kg
rel. Standard deviaton	15,4	%
Horwitz standard deviation	10,2	%
HorRat-value	**1,5**	
Recovery rate	85	%

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		Germany
		SWITZERLAND
		CANADA
		ITALY
		Germany
		Germany
		SWEDEN
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN
		Germany
		Germany
		Germany
		AUSTRIA
		Germany

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
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4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
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15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
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