Evaluation Report
proficiency test

DLA ptAL05 (2021)

Allergens V:

Hazelnut, Walnut and Egg

in Pastry (Cocoa Biscuit)

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

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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Contents

1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is cocoa biscuits baked by DLA. The basic composition was the same for both samples A and B (see Table 1).
After crushing and sieving using an impact mill (mesh 1,5 mm), the basic mixture was homogenized.
The **spiked sample A** was then produced as follows:
As a further ingredient, biscuits were baked with the spiking material (150°C, 30 min), which contains the allergenic ingredients hazelnut, walnut and egg, and then dried (50°C, overnight). After crushing, sieving (1,5 mm mesh) and homogenization, the baked biscuit containing the allergenic ingredients was added to an aliquot of the basic matrix and the mixture was homogenized. Subsequently, the basic mixture was again added in additional steps and homogenized in each case until the total quantity had been reached.
The **spiking level sample** was produced with the above-mentioned allergen-containing spiking material(s) with multi-stage addition of potato powder (mesh 500 µm) and homogenization.

Samples A and B were filled in portions of approx. 25 g and the spiking level sample of approx. 15 g in metallized PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Cocoa Biscuits, gluten-free (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4,6%), rice protein, salt	96,0 g/100g	100 g/100g	-
Cocoa Biscuits, spiked (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4,6%), rice protein, salt, as well as hazelnut, walnut, egg and other ingredients (see below)	4,0 g/100g	-	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,9 g/100g
Hazelnuts, roasted ground, mixture (10 products from 5 countries, Europe) - as hazelnut* - *thereof 14,1% total protein***	 19,8 mg/kg 2,79 mg/kg	-	 12,1 mg/kg 1,70 mg/kg
Walnuts, roasted ground, mixture (11 products i.a. from North America, South America, Europe) - as walnut* - *thereof 15,8% total protein***	 20,4 mg/kg 3,22 mg/kg	-	 12,0 mg/kg 1,88 mg/kg
Whole egg powder pasteurized, spray dried, mixture (6 products from 2 countries, Europe) - as whole egg powder* - thereof 46,9% total protein** - thereof 26,0% egg white protein***	 20,6 mg/kg 9,67 mg/kg 5,36 mg/kg	-	 13,0 mg/kg 6,10 mg/kg 3,38 mg/kg
Further Ingredients: *Maltodextrin and silicon dioxide*	<0,2 g/100 g	-	<0,2 g/100 g

* Allergen contents as "total food" as described in the column ingredients according to the gravimetric mixture
** Protein contents according to laboratory analysis of the raw material (total nitrogen according to Kjeldahl with F=5,30 for hazelnut and walnut protein and with F=6,25 for whole egg protein)
*** Egg white protein according to literature [36, 37]

Note: The metrological traceability of temperature, mass and volume during the 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 showed a probability of 74% and 72%, 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]. HorRat values of 1,0 each were obtained. 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 (exception: Morinaga ELISA II performed by DLA). 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 hazelnut (Immunolab, Morinaga and AgraQuant), walnut (Immunolab and AgraQuant) and egg (Morinaga) (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].

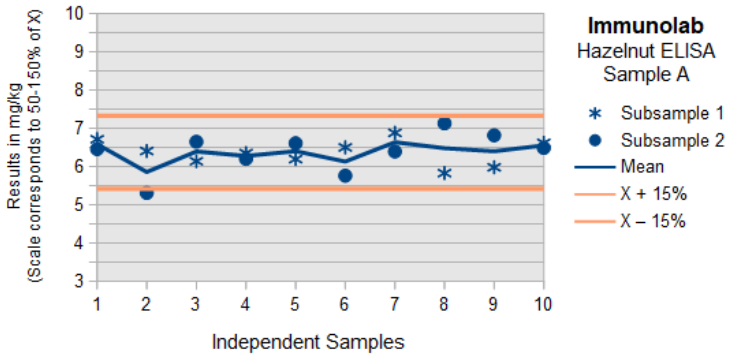
ELISA-Tests: Homogenität Haselnuss / Homogeneity Hazelnut

Immunolab Hazelnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut 6,37 ± 0,23 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,71	6,45	6,58
2	6,40	5,31	5,85
3	6,14	6,64	6,39
4	6,35	6,20	6,27
5	6,19	6,61	6,40
6	6,50	5,76	6,13
7	6,88	6,39	6,63
8	5,82	7,13	6,48
9	5,98	6,81	6,40
10	6,62	6,48	6,55

General average X	6,37	
SD of sample means Sx	0,23	3,7%
SD w ithin-samples Sw	0,35	5,5%
SD betw een-samples Ss	< 0,23	< 3,7%

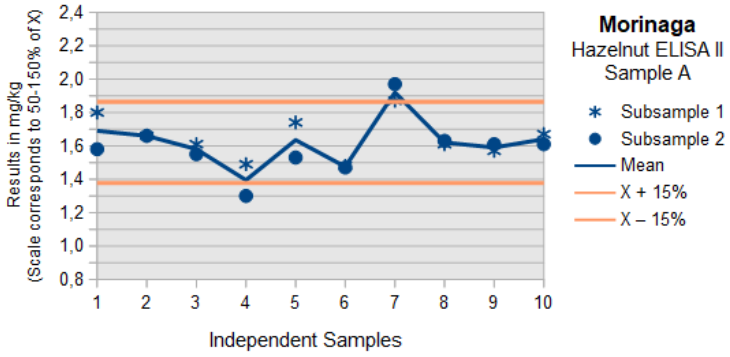


Morinaga Hazelnut ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut Protein 1,62 ± 0,13 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	1,80	1,58	1,69
2	1,66	1,66	1,66
3	1,61	1,55	1,58
4	1,49	1,30	1,40
5	1,74	1,53	1,64
6	1,48	1,47	1,48
7	1,87	1,97	1,92
8	1,61	1,63	1,62
9	1,57	1,61	1,59
10	1,67	1,61	1,64

General average X	1,62	
SD of sample means Sx	0,14	8,5%
SD w ithin-samples Sw	0,06	3,8%
SD betw een-samples Ss	0,13	8,1%

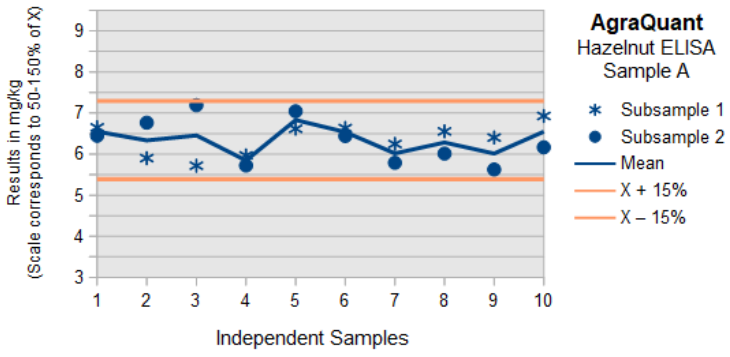


AgraQuant Hazelnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut 6,34 ± 0,18 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,65	6,45	6,55
2	5,90	6,76	6,33
3	5,72	7,19	6,45
4	5,97	5,72	5,85
5	6,62	7,04	6,83
6	6,64	6,44	6,54
7	6,25	5,79	6,02
8	6,55	6,01	6,28
9	6,40	5,63	6,01
10	6,93	6,17	6,55

General average X	6,34	
SD of sample means Sx	0,30	4,8%
SD w ithin-samples Sw	0,35	5,5%
SD betw een-samples Ss	0,18	2,8%



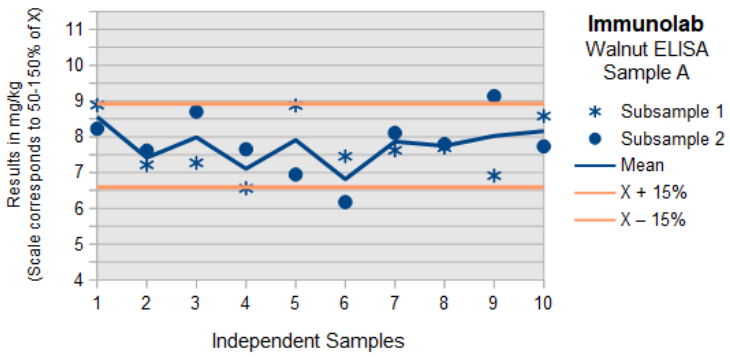
ELISA-Tests: Homogenität Walnuss / Homogeneity Walnut

Immunolab Walnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Walnut 7,76 ± 0,28 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	8,89	8,22	8,56
2	7,21	7,61	7,41
3	7,27	8,70	7,99
4	6,56	7,65	7,11
5	8,87	6,95	7,91
6	7,45	6,18	6,82
7	7,62	8,11	7,87
8	7,70	7,80	7,75
9	6,91	9,14	8,02
10	8,58	7,73	8,16

General average X	7,76	
SD of sample means Sx	0,51	6,6%
SD within-samples Sw	0,61	7,9%
SD between-samples Ss	0,28	3,6%

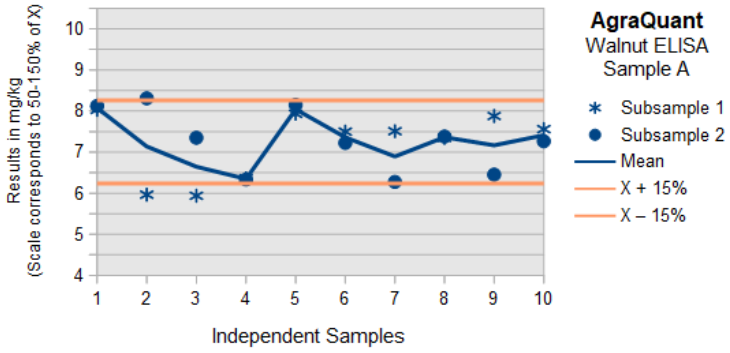


AgraQuant Walnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Walnut 6,74 ± 0,40 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	7,53	7,62	7,58
2	5,46	7,81	6,64
3	5,44	6,85	6,14
4	5,86	5,83	5,85
5	7,43	7,65	7,54
6	7,00	6,72	6,86
7	7,01	5,78	6,39
8	6,85	6,88	6,86
9	7,38	5,95	6,67
10	7,06	6,76	6,91

General average X	6,74	
SD of sample means Sx	0,55	8,1%
SD within-samples Sw	0,53	7,9%
SD between-samples Ss	0,40	5,9%



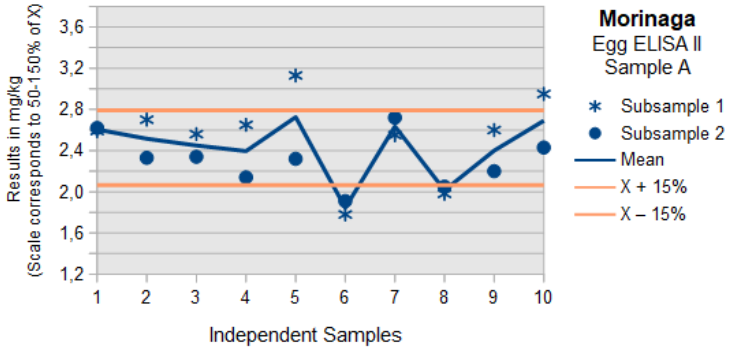
ELISA-Tests: Homogenität Ei / Homogeneity Egg

Morinaga Egg ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Egg Protein 2,43 ± 0,25 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	2,59	2,62	2,61
2	2,70	2,33	2,52
3	2,56	2,34	2,45
4	2,65	2,14	2,40
5	3,13	2,32	2,73
6	1,78	1,91	1,85
7	2,55	2,72	2,64
8	1,98	2,05	2,02
9	2,60	2,20	2,40
10	2,95	2,43	2,69

General average X	2,43	
SD of sample means Sx	0,29	11,9%
SD within-samples Sw	0,20	8,2%
SD between-samples Ss	0,25	10,4%



2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,32 (20,0°C) and 0,34 (20,5°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 34th week of 2021. The testing method was optional. The tests should be finished at 22nd October 2021 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters Hazelnut, Walnut and Egg in the range of mg/kg in the matrix of Cocoa biscuits. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing and should be analysed like a normal sample.

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

2.3 Submission of results

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

30 out of 32 participants submitted at least one result. Two participants did not submit any results.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [25, 26, 27, 28]. It is for this reason that we contrast the results of the present proficiency test with several assigned values.
Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.

For quantitative results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].
In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Also, 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. This results are checked by kernel density estimation [3, 12].

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

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

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

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

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-35]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Peanut	Rice biscuits	23,4 5,19	113 % 99,7 %	15,6% 15,0%	11,6% 14,7%	14,4% 18,1%	11,8% 14,8%	rt-PCR ASU 00.00-169
Peanut	Wheat biscuits (DLA)	1,97	39,3 %	16,2%	16,0%	19,5%	15,8%	rt-PCR ASU 00.00-169
Peanut	Milk powder Boiled sausage	3,66 2,44	73,2 % 49,4 %	15,8% 15,6%	12,8% 11,9%	14,8% 15,9%	11,7% 13,5%	rt-PCR ASU 00.00-169
Almond	Rice biscuits	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 biscuits 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 biscuits	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 biscuits 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 biscuits	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 biscuits 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 biscuits	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 biscuits 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 evaluation the z-scores below are calculated with a target standard deviation of 25%:

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

3.5.1 Warning and action signals

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

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

ELISA results, which were given as **hazelnut protein**, have been converted to the **total food (hazelnut)** using the experimentally determined protein content of the raw materials (see p. 5).

ELISA results reported as **egg white proteins** or **egg protein (egg white and egg yolk proteins)** have been converted to **whole egg powder**. If stated, the specifications of the test kit manufacturer concerned have been taken into account. A proportion of 26% egg white protein in whole egg powder was taken as the basis. Total egg protein results (Moringa and Moringa Kit II) were converted to the total food (whole egg powder) using the experimentally determined protein content of the raw materials (see p. 5).

No conversions were necessary for the parameter walnut.

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.

If 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})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{METHOD\ i}}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* σ_{pt}'		
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}'$)°		
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}'$)°		
Quotient S*/σ_{pt} *or* S*/σ_{pt}'		
Standard uncertainty $U_{(X_{pt})}$		
Number of results in target range		
Percent in target range		

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

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

4.1 Proficiency Test Hazelnut

4.1.1 ELISA Results: Hazelnut

Qualitative evaluation of the 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		
19	positive	5,00	negative	<LOQ	2/2 (100%)	AQ	
21	positive	6,23	negative	<1	2/2 (100%)	AQ	
25	positive	6,50	negative	<1	2/2 (100%)	AQ	
13	positive	6,20	negative	<1	2/2 (100%)	BF	
12	positive	5,26	negative		2/2 (100%)	IL	
23	positive	6,20	negative	<1	2/2 (100%)	IL	
30	positive	4,10	negative	<1	2/2 (100%)	IL	
4	positive	6,67	negative	<1,1	2/2 (100%)	MI-II	result converted °
5	positive	8,20	negative	<2,5	2/2 (100%)	RS-F	
6	positive	9,86	negative	<2.5	2/2 (100%)	RS-F	
7	positive	14,4	negative	<2.5	2/2 (100%)	RS-F	
9	positive	12,3	negative		2/2 (100%)	RS-F	
10	positive	8,90	negative	<2,5	2/2 (100%)	RS-F	
16	positive	13,5	negative	<0,2	2/2 (100%)	RS-F	
17	positive	10,6	negative		2/2 (100%)	RS-F	
18	positive	12,3	negative	<2.5	2/2 (100%)	RS-F	
20	positive	10,9	negative	<2,5	2/2 (100%)	RS-F	
24	positive	12,9	negative	<2,5	2/2 (100%)	RS-F	
27	positive	13,7	negative	<2.5	2/2 (100%)	RS-F	
28	positive	11,0	negative	<2,5	2/2 (100%)	RS-F	
1	positive	9,00	negative	0	2/2 (100%)	SP	
2	positive	5,20	negative	<1.0	2/2 (100%)	SP	
14	positive	6,50	negative	< 2,50	2/2 (100%)	VT	
22	positive	6,80	negative	<2,5	2/2 (100%)	VT	
26	positive	6,00	negative	<2.5	2/2 (100%)	VT	
29	positive	7,60	negative	0	2/2 (100%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	26		0	
Number negative	0		26	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative evaluation of ELISA-results: Sample A

Evaluation number	Hazelnut	z-Score $Xpt_{Peak\ 6}$	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
19	5,00	-0,76		AQ	
21	6,23	0,03		AQ	
25	6,50	0,21		AQ	
13	6,20	0,01		BF	
12	5,26	-0,60		IL	
23	6,20	0,01		IL	
30	4,10	-1,3		IL	
4	6,67	0,32		MI-II	result converted °
5	8,20		-1,2	RS-F	
6	9,86		-0,59	RS-F	
7	14,4		0,99	RS-F	
9	12,3		0,26	RS-F	
10	8,90		-0,92	RS-F	
16	13,5		0,66	RS-F	
17	10,6		-0,33	RS-F	
18	12,3		0,27	RS-F	
20	10,9		-0,23	RS-F	
24	12,9		0,48	RS-F	
27	13,7		0,75	RS-F	
28	11,0		-0,19	RS-F	
1	9,00	1,8		SP	
2	5,20	-0,63		SP	
14	6,50	0,21		VT	
22	6,80	0,40		VT	
26	6,00	-0,12		VT	
29	7,60	0,92		VT	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

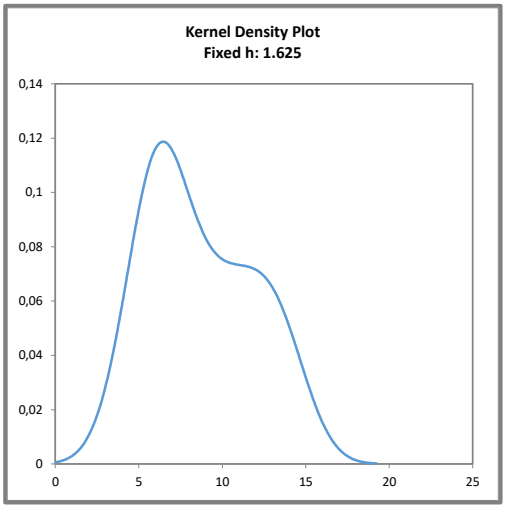


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an approximately symmetrical distribution of the results of the main peak "Peak 6" at approx. 6 mg/kg and a secondary peak at approx. 12 mg/kg, which is based on the results of method RS-F.

Characteristics: Quantitative evaluation ELISA Hazelnut

Sample A

Statistic Data	**Methods Peak 6** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{Peak\ 6}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	14	12
Number of outliers	0	0
Mean	6,23	11,5
Median	6,22	11,7
Robust Mean (X_{pt})	**6,18**	**11,6**
Robust standard deviation (S*)	**1,04**	**2,22**
Target range:		
Target standard deviation σ_{pt}	**1,55**	**2,89**
lower limit of target range	**3,09**	**5,78**
upper limit of target range	**9,27**	**17,3**
Quotient S/σ_{pt}*	*0,67*	*0,77*
Standard uncertainty $U_{(Xpt)}$	*0,348*	*0,800*
Results in the target range	*14*	*12*
Percent in the target range	*100*	*100*

Methods:
Peak 6 = AgraQuant, Biofront, Immunolab, Morinaga, SensiSpec, Veratox
RS-F (Peak 12) = Ridascreen® Fast, R-Biopharm

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed a bimodal distribution of the results. Therefore, no joint evaluation of all methods was carried out, but a separate evaluation of the methods that are to be assigned to the main peak ("Peak 6") and the secondary peak (method RS-F) (for assignment see above under the table).

The evaluations of the results of the methods of "Peak 6" and of method RS-F showed a low variability of the results. The quotient S/σ_{pt} was below 1,0 in each case. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (cf. 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 31% (methods "Peak 6") and 59% (method RS-F) of the spiking level of hazelnut to sample A and thus below or within the range of the recommendations for the applied methods (see 3.4.3 and p.32 "Recovery rates with z-scores ELISA for Hazelnut").

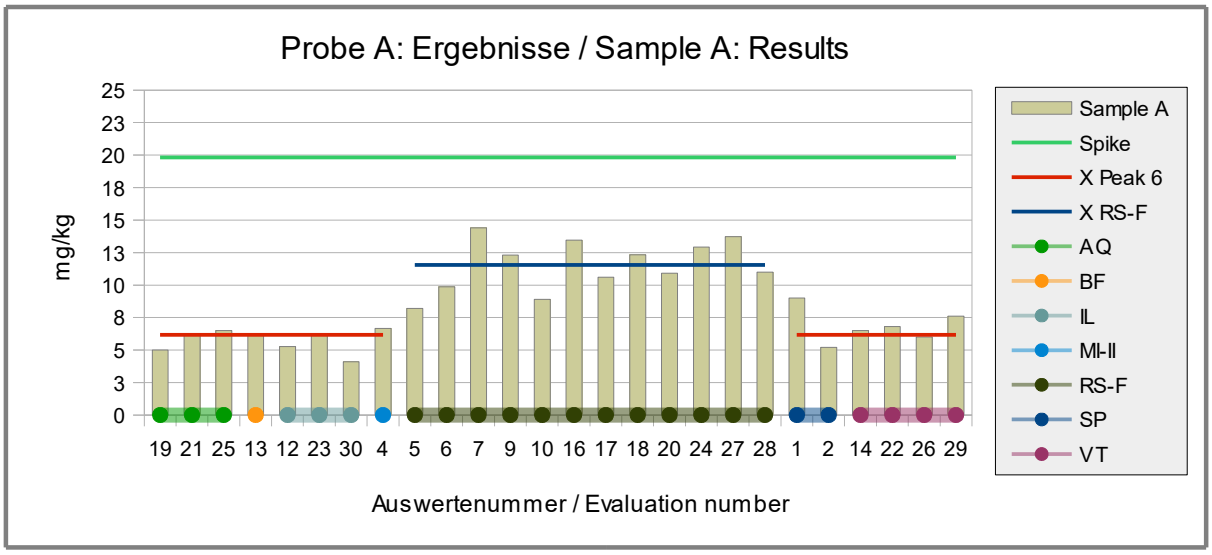


Abb./Fig. 2: ELISA Results Hazelnut
green line = Spiking level (Spike)
red line = Assigned value robust mean "Peak 6"
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

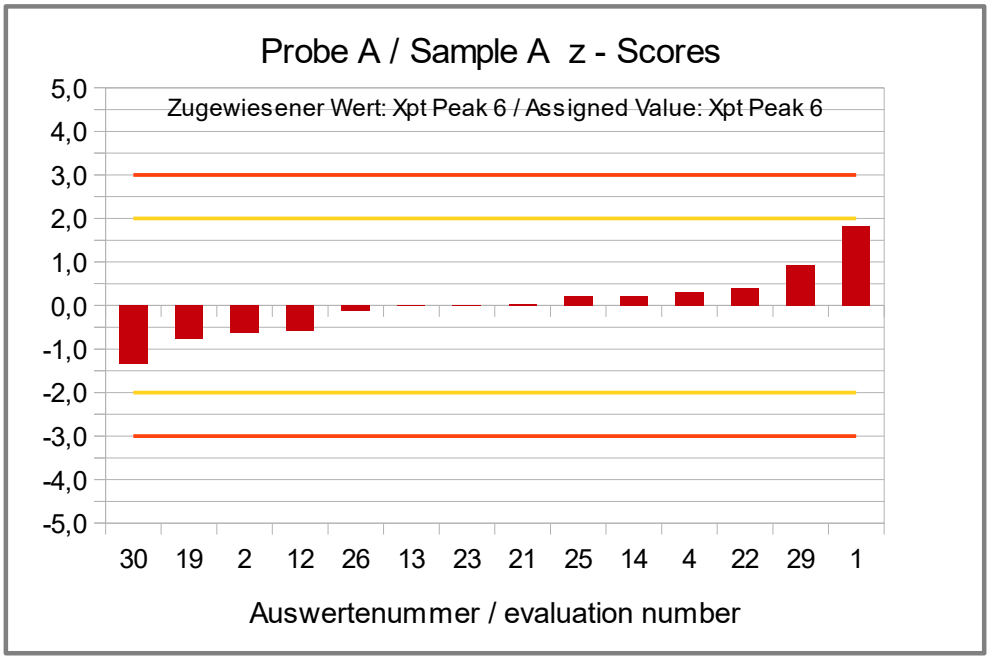


Abb./Fig. 3:
z-Scores (ELISA Results Hazelnut)
Assigned value robust mean of all results of "Peak 6"

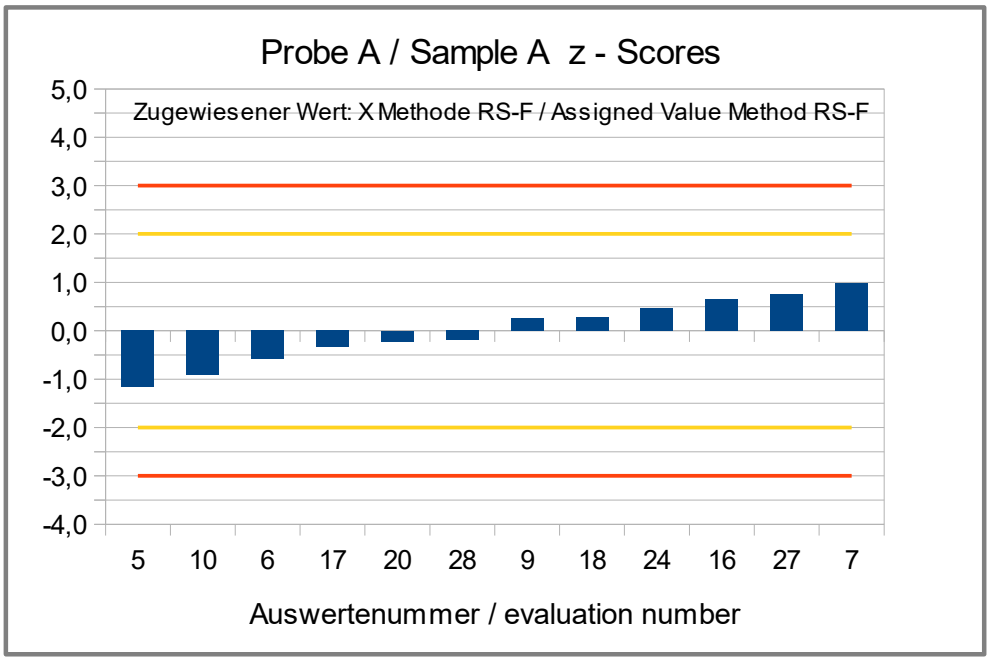


Abb./Fig. 4:
z-Scores (ELISA Results Hazelnut)
Assigned value robust mean of method RS-F (Ridascreen® Fast, R-Biopharm)

Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Hazelnut	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
19	10,0	-0,16		AQ	
21	10,4	0,01		AQ	
25	9,40	-0,39		AQ	
13	17,9	2,9		BF	
12	7,81	-1,0		IL	
23	9,10	-0,50		IL	
30	2,70	-3,0		IL	
4	5,82	-1,8		MI-II	result converted °
5	10,9	0,19	-0,40	RS-F	
6	15,1	1,8	0,98	RS-F	
7	13,2	1,1	0,36	RS-F	
9	12,5	0,80	0,13	RS-F	
10	2,50	-3,0	-3,2	RS-F	
16	14,5	1,6	0,78	RS-F	
17	8,60	-0,70	-1,2	RS-F	
18	13,9	1,3	0,59	RS-F	
20	9,80	-0,23	-0,77	RS-F	
24	13,2	1,1	0,34	RS-F	
27	13,6	1,2	0,48	RS-F	
28	12,0	0,61	-0,04	RS-F	
1	11,0	0,23		SP	
2	12,0	0,61		SP	
14	6,55	-1,5		VT	
22	7,50	-1,1		VT	
26	10,4	0,00		VT	
29	7,40	-1,2		VT	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

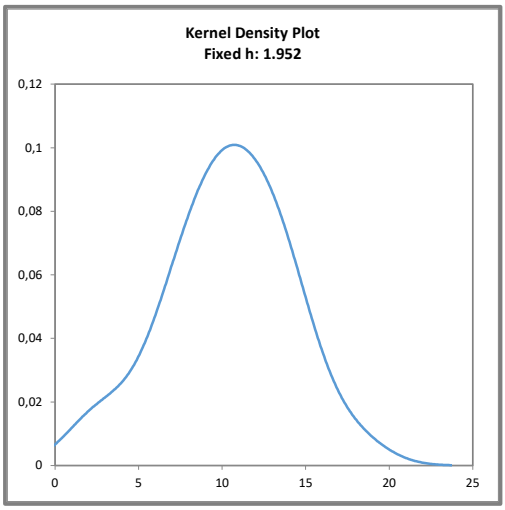


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an approximately symmetrical distribution of the results with a slight shoulder at < 5 mg/kg.

Characteristics: Quantitative evaluation ELISA Hazelnut

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	26	12
Number of outliers	0	–
Mean	10,3	11,6
Median	10,4	12,8
Robust Mean (X_{pt})	**10,4**	**12,1**
Robust standard deviation (S*)	**3,47**	**2,56**
Target range:		
Target standard deviation σ_{pt}	**2,60**	**3,03**
lower limit of target range	**5,20**	**6,06**
upper limit of target range	**15,6**	**18,2**
Quotient S/σ_{pt}*	*1,3*	*0,84*
Standard uncertainty $U_{(Xpt)}$	*0,851*	*0,923*
Results in the target range	*23*	*11*
Percent in the target range	*88*	*92*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed an approximately symmetrical distribution of the results without clear method-dependent differences.

The distribution of the results for all methods as well as for method RS-F showed a normal or low variability. The quotients S/σpt were below 2,0 and below 1,0, respectively. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (cf. 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 86% and 100% of the spiking level of hazelnut to the spiking level sample and were thus within the relevant requirements for the methods used (see 3.4.3 and page 32 "Recovery rates with z-scores ELISA for Hazelnut").

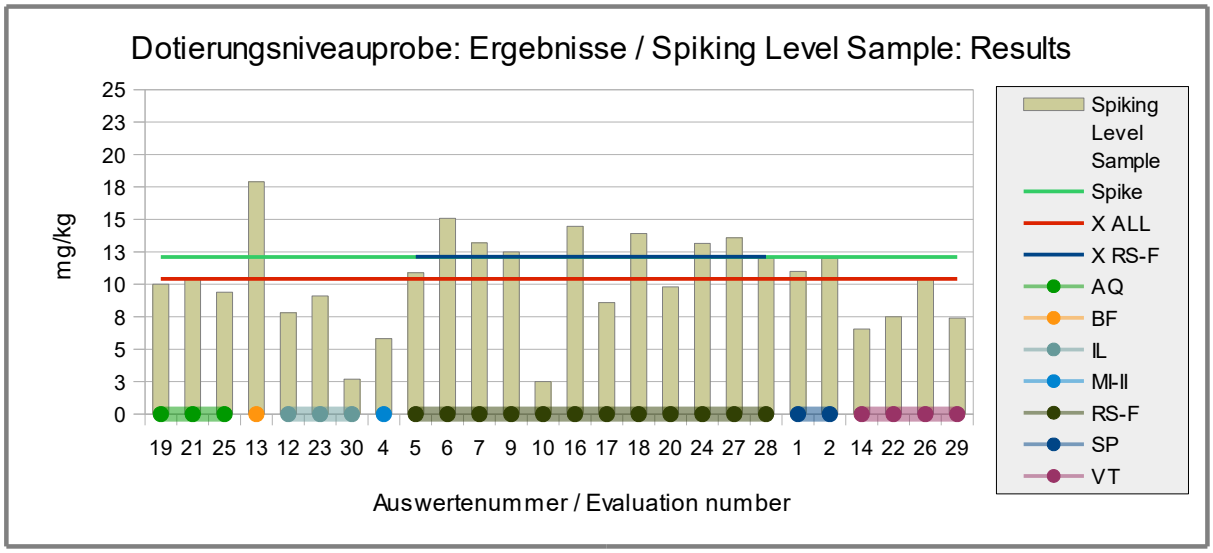


Abb./Fig. 6: ELISA Results Hazelnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

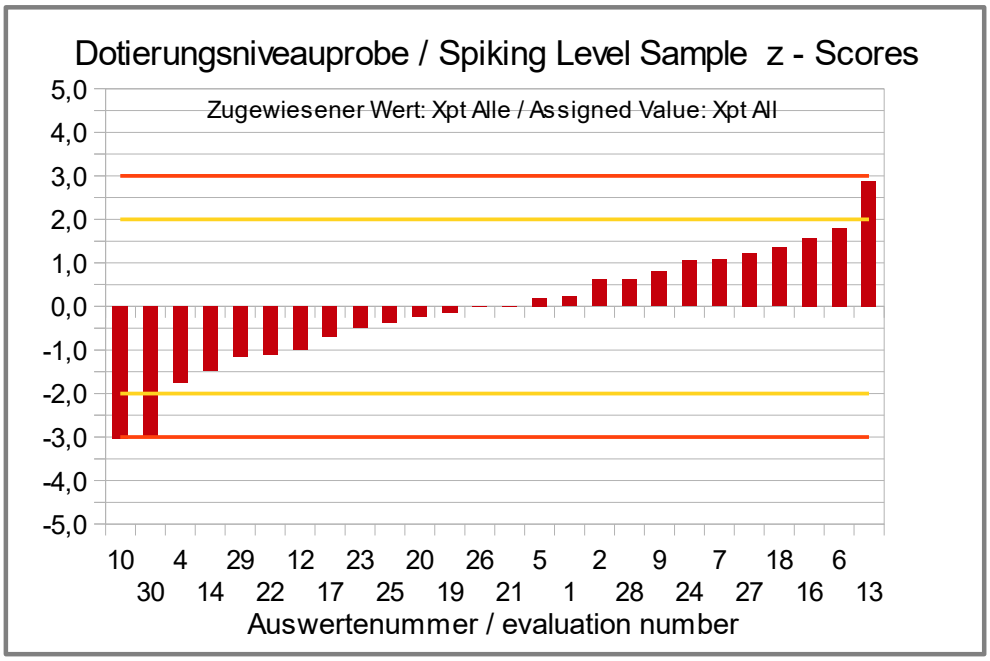


Abb./Fig. 7:
z-Scores (ELISA Results Hazelnut)
Assigned value robust mean of all results

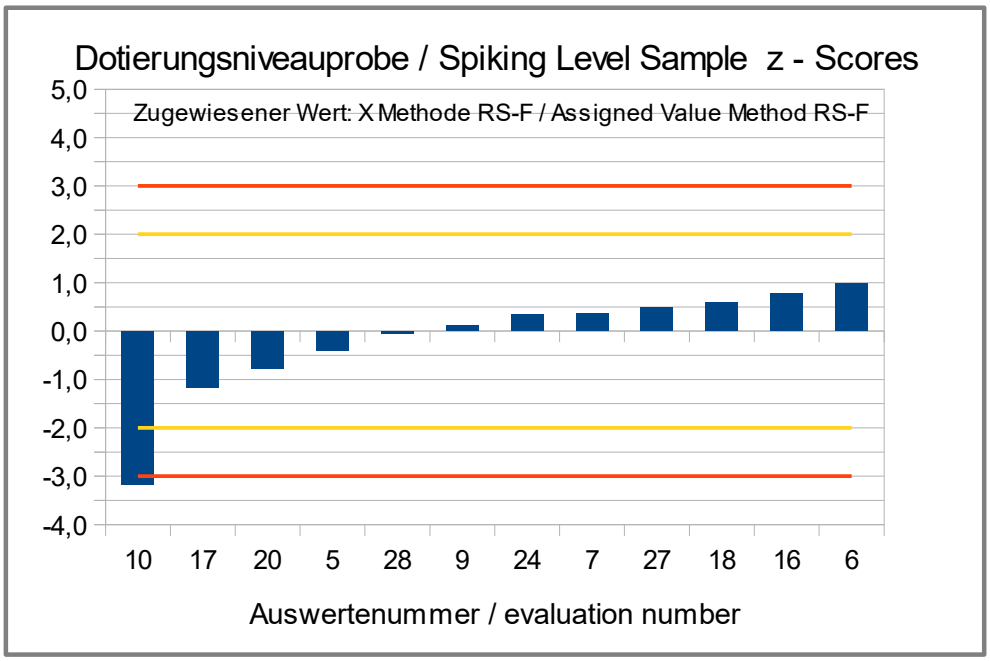


Abb./Fig. 8:
z-Scores (ELISA Results Hazelnut)
Assigned value robust mean of method RS-F (Ridascreen® Fast, R-Biopharm)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
19	10,0	**83**	-0,69	5,00	25	-3,0	AQ	
21	10,4	**86**	-0,55	6,23	31	-2,7	AQ	
25	9,40	**78**	-0,89	6,50	33	-2,7	AQ	
13	17,9	**148**	1,9	6,20	31	-2,7	BF	
12	7,81	**65**	-1,4	5,26	27	-2,9	IL	
23	9,10	**75**	-1,0	6,20	31	-2,7	IL	
30	2,70	22	-3,1	4,10	21	-3,2	IL	
4	5,82	48	-2,1	6,67	34	-2,7	MI-II	result converted °
5	10,9	**90**	-0,40	8,20	41	-2,3	RS-F	
6	15,1	**125**	1,0	9,86	**50**	-2,0	RS-F	
7	13,2	**109**	0,36	14,4	**73**	-1,1	RS-F	
9	12,5	**103**	0,13	12,3	**62**	-1,5	RS-F	
10	2,50	21	-3,2	8,90	45	-2,2	RS-F	
16	14,5	**120**	0,78	13,5	**68**	-1,3	RS-F	
17	8,60	**71**	-1,2	10,6	**53**	-1,9	RS-F	
18	13,9	**115**	0,60	12,3	**62**	-1,5	RS-F	
20	9,80	**81**	-0,76	10,9	**55**	-1,8	RS-F	
24	13,2	**109**	0,35	12,9	**65**	-1,4	RS-F	
27	13,6	**112**	0,49	13,7	**69**	-1,2	RS-F	
28	12,0	**99**	-0,03	11,0	**55**	-1,8	RS-F	
1	11,0	**91**	-0,36	9,00	45	-2,2	SP	
2	12,0	**99**	-0,03	5,20	26	-3,0	SP	
14	6,55	**54**	-1,8	6,50	33	-2,7	VT	
22	7,50	**62**	-1,5	6,80	34	-2,6	VT	
26	10,4	**86**	-0,56	6,00	30	-2,8	VT	
29	7,40	**61**	-1,6	7,60	38	-2,5	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	23	Number in RA	10
Percent in RA	88	Percent in RA	38

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

For the spiking level sample 88% (23) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 38% (10) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.1.2 PCR Results: Hazelnut

Qualitative evaluation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
3	positive		negative		2/2 (100%)	SFA	
5	positive		negative		2/2 (100%)	SFA	
13	positive		negative		2/2 (100%)	SFA	
15	positive		negative		2/2 (100%)	SFA	
27	positive	3,13	negative	<1	2/2 (100%)	SFA	
8	positive		negative		2/2 (100%)	SFA-4p	
11	negative		negative		1/2 (50%)	SFA-4p	no positive sample identified

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

Quantitative evaluation of PCR Results: Sample A

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

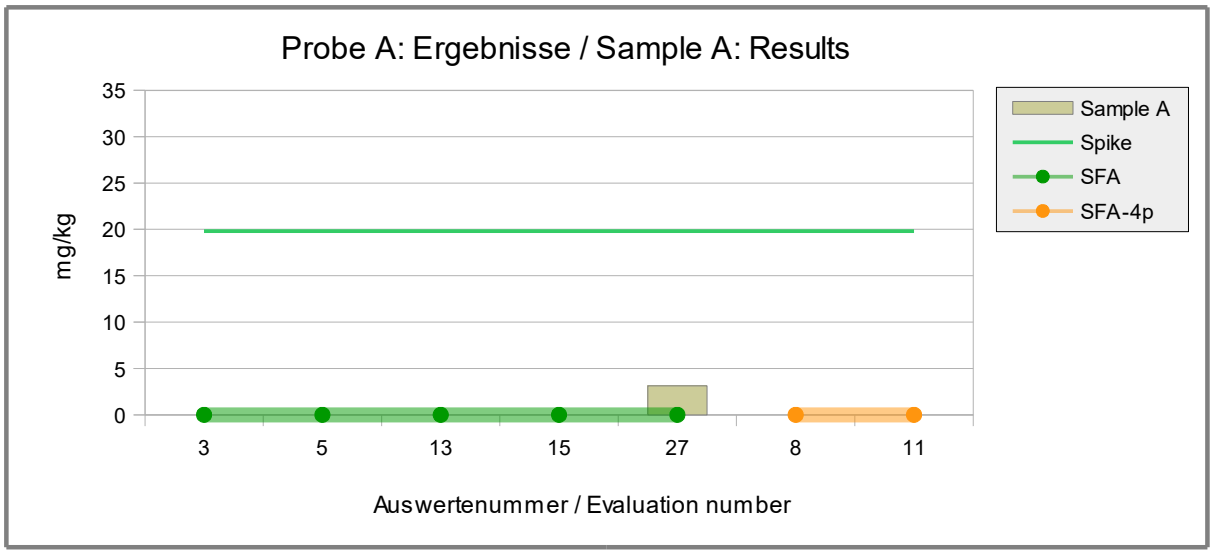


Abb./Fig. 9: PCR Results Hazelnut
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Qualitative evaluation of PCR Results: Spiking Level Sample

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

Evaluation number	Hazelnut	Hazelnut	Z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
3	positive			SFA	
5	positive			SFA	
13	positive			SFA	
15	positive			SFA	
27	positive	12,1		SFA	
8	positive			SFA-4p	
11	positive			SFA-4p	

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

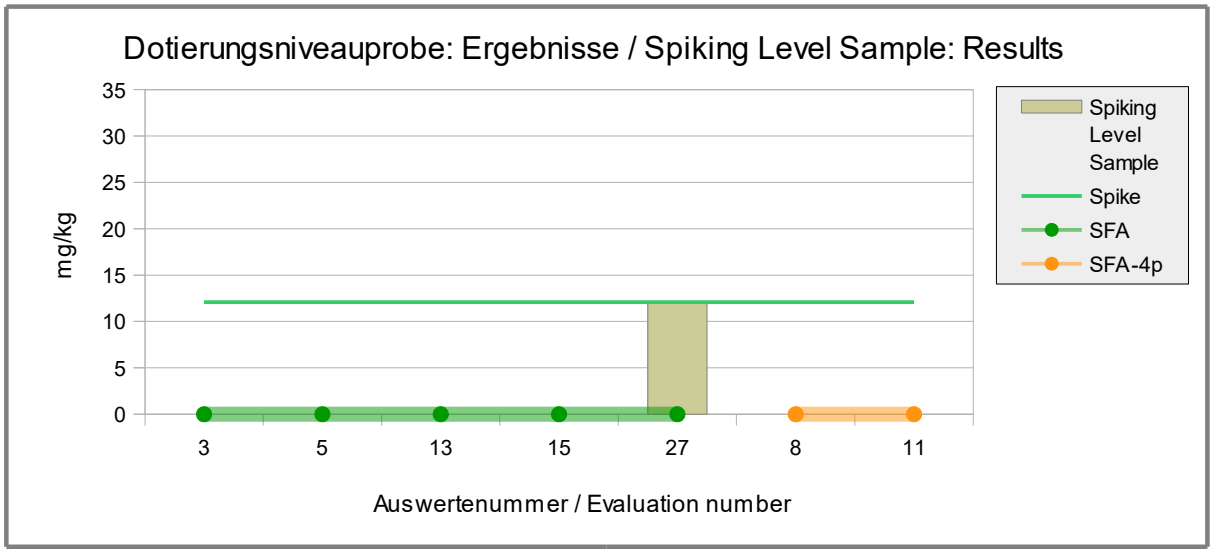


Abb./Fig. 10: *PCR Results Hazelnut*
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
3							SFA	
5							SFA	
13							SFA	
15							SFA	
27	12,1	**100**	0,01	3,13	16	-3,4	SFA	
8							SFA-4p	
11							SFA-4p	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	0
Percent in RA	100	Percent in RA	0

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
One participant submitted results by PCR methods and obtained a recovery rate within the range of the AOAC-recommendation of 50-150% for the spiking level sample.
The related z-scores are based on the target standard deviation of 25%.

4.2 Proficiency Test Walnut

4.2.1 ELISA Results: Walnut

Qualitative evaluation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
7	negative	<2,0	negative	<2,0	1/2 (50%)	AQ	no positive sample identified
14	positive	10,6	negative	< 6,0	2/2 (100%)	AQ	
17	positive	8,50	negative		2/2 (100%)	AQ	
19	positive	9,00	negative	<LOQ	2/2 (100%)	AQ	
25	positive	3,80	negative	<2	2/2 (100%)	AQ	
21	positive	4,25	negative	<2	2/2 (100%)	BC	
27	positive	5,00	negative	<2	2/2 (100%)	BC	
10	positive	9,50	negative	<2	2/2 (100%)	BF	
13	positive	9,03	negative	<1	2/2 (100%)	BF	
20	positive	10,6	negative	<1,0	2/2 (100%)	BF	
26	positive	11,6	negative	<2,0	2/2 (100%)	BF	
6	positive	17,9	negative	<2,4	2/2 (100%)	BK	
29	positive	15,8	negative	0	2/2 (100%)	BK	
12	positive	5,68	negative		2/2 (100%)	IL	
23	positive	6,10	negative	<2	2/2 (100%)	IL	
30	positive	3,20	negative	<2	2/2 (100%)	IL	
16	positive	9,60	negative	<0,6	2/2 (100%)	NL	
1	positive	9,00	negative	0	2/2 (100%)	SP	
2	positive	8,40	negative	<2,0	2/2 (100%)	SP	
4	positive	7,50	negative	<2	2/2 (100%)	SP	
5	positive	7,70	negative	<2,0	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	20		0	
Number negative	1		21	
Percent positive	95		0	
Percent negative	5		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
SP = SensiSpec ELISA Kit, Eurofins
div = not indicated / other method

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

Quantitative evaluation of ELISA-results: Sample A (informative)

The following evaluation was done solely for information

Evaluation number	Walnut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]	Info		
7	<2,0		AQ	
14	10,6	1,0	AQ	
17	8,50	0,05	AQ	
19	9,00	0,28	AQ	
25	3,80	-2,2	AQ	
21	4,25	-2,0	BC	
27	5,00	-1,6	BC	
10	9,50	0,52	BF	
13	9,03	0,30	BF	
20	10,6	1,0	BF	
26	11,6	1,5	BF	
6	17,9	4,5	BK	
29	15,8	3,5	BK	
12	5,68	-1,3	IL	
23	6,10	-1,1	IL	
30	3,20	-2,5	IL	
16	9,60	0,57	NL	
1	9,00	0,28	SP	
2	8,40	0,00	SP	
4	7,50	-0,43	SP	
5	7,70	-0,34	div	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
SP = SensiSpec ELISA Kit, Eurofins
div = not indicated / other method

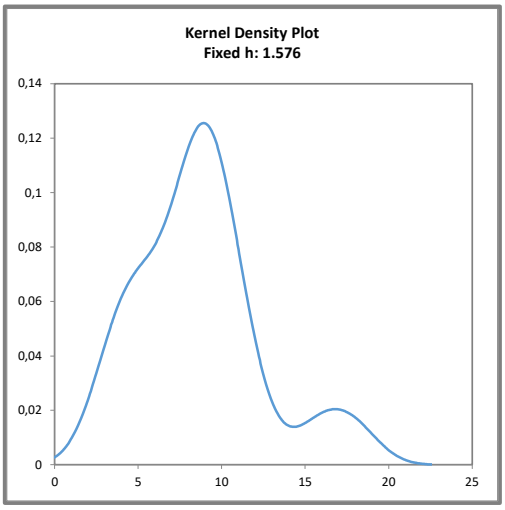


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an approximately symmetrical distribution of the results with a shoulder at approx. < 7 mg/kg (methods BC and IL) and a secondary peak at approx. 17 mg/kg (method BK).

Characteristics: Quantitative evaluation ELISA Walnut

The following evaluation was done solely for information

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	20
Number of outliers	0
Mean	8,64
Median	8,75
Robust Mean (X_{pt})	**8,41**
Robust standard deviation (S*)	**3,24**
Target range:	
Target standard deviation σ_{pt}	**2,10**
lower limit of target range	**4,20**
upper limit of target range	**12,6**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*0,906*
Results in the target range	*16*
Percent in the target range	*80*

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed an approximately symmetrical distribution of the results for the main peak with indications of possible method-dependent differences. The joint cross-method evaluation is therefore purely informative. The resulting target range is not valid for the single methods. Since there were < 5 single results per method, no separate evaluations were carried out.

The evaluation of the results of all methods showed a normal variability of the results. The quotient S/σ_{pt} was below 2.0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (cf. 3.4.2 value by precision experiments and 3.4.3 value by perception).*

The robust mean of the evaluation was 41% of the spiking level of walnut to sample A and thus below the range of the recommendations for the applied methods (see 3.4.3 and p. 45 "Recovery rates with z-scores ELISA for Walnut").

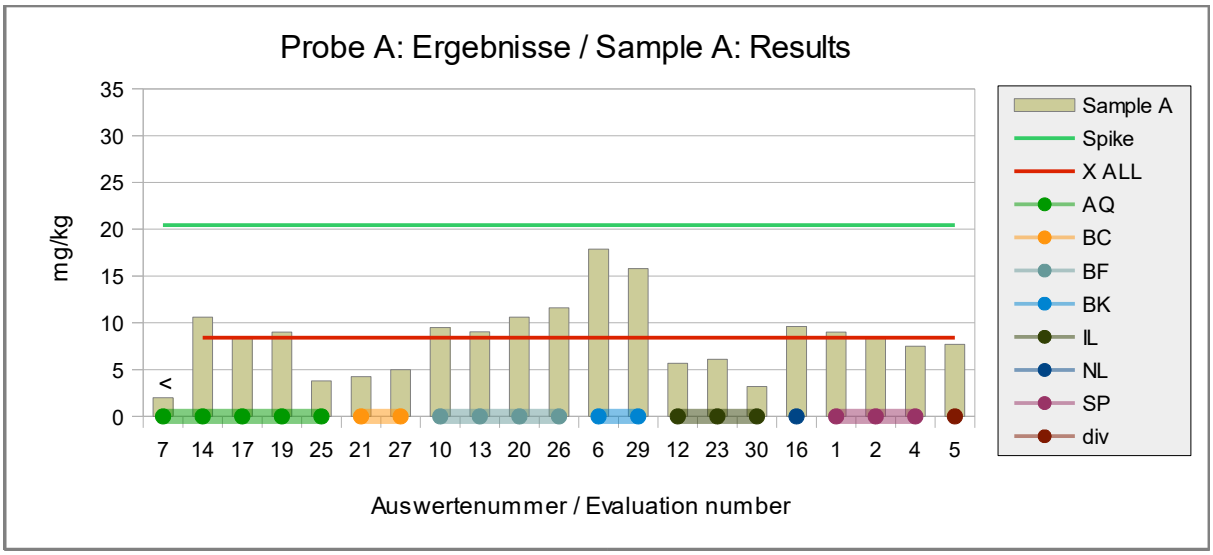


Abb./Fig. 12: ELISA Results Walnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

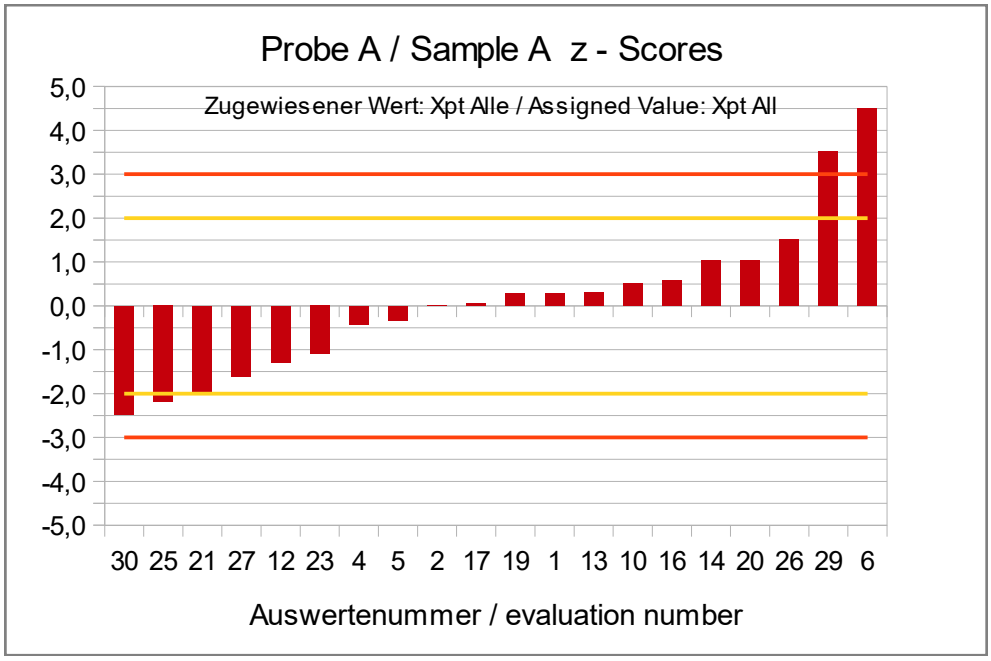


Abb./Fig. 13:
z-Scores for information (ELISA Results Walnut)
Assigned value robust mean of all results

Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Walnut	z-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
7	26,8	-1,1	-1,6	AQ	
14	56,2	2,1	1,1	AQ	
17	42,4	0,62	-0,19	AQ	
19	55,0	2,0	0,95	AQ	
25	41,9	0,56	-0,23	AQ	
21	35,7	-0,11		BC	
27	41,5	0,53		BC	
10	5,00			BF	
13	18,3			BF	
20	13,6			BF	
26	14,4			BF	
6	72,5	3,9		BK	
29	38,4	0,18		BK	
12	64,4	3,0		IL	
23	51,8	1,6		IL	
30	9,00	-3,0		IL	
16	35,0	-0,19		NL	
1	>60			SP	
2	43,1	0,69		SP	
4	35,0	-0,19		SP	
5	38,4	0,18		div	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
SP = SensiSpec ELISA Kit, Eurofins
div = not indicated / other method

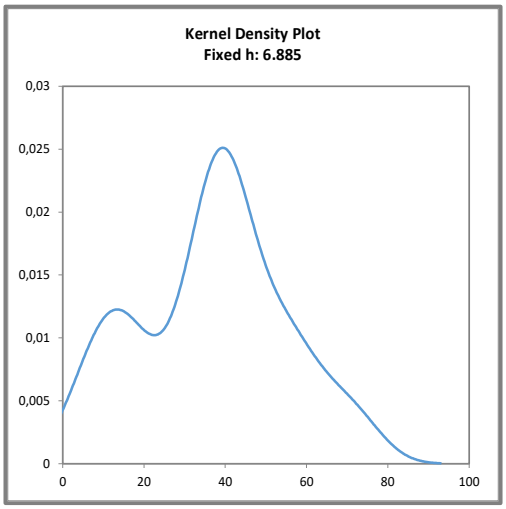


Abb. / Fig. 14:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetric distribution of the main peak with a secondary peak at approx. 17 mg/kg (method BF) and a broad course at about > 55 mg/kg.

Characteristics: Quantitative evaluation ELISA Walnut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	16°	5
Number of outliers	4	0
Mean	42,9	44,5
Median	41,7	42,4
Robust Mean (X_{pt})	**43,2**	**44,5**
Robust standard deviation (S*)	**13,4**	**13,6**
Target range:		
Target standard deviation σ_{pt}	**10,8**	**11,1**
lower limit of target range	**21,6**	**22,2**
upper limit of target range	**64,8**	**66,7**
Quotient S/σ_{pt}*	*1,2*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*4,20*	*7,58*
Results in the target range	*14*	*5*
Percent in the target range	*88*	*100*

° *without results No. 10, 13, 20 und 26 (excluded in advance)*

Methods:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed method-dependent differences with respect to method BF, which was therefore excluded from the evaluation. Since < 5 single results were available for this method, no separate evaluation was carried out.

The distribution of the results for all methods (without BF) as well as for method AQ showed a normal variability in each case. The quotients S/σpt were below 2.0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 361% and 372% of the spiking level of walnut to the spiking level sample and were thus clearly above the range of the recommendations for the applied methods (s. 3.4.3 and p. 45 "Recovery rates with z-Scores ELISA for Walnut").

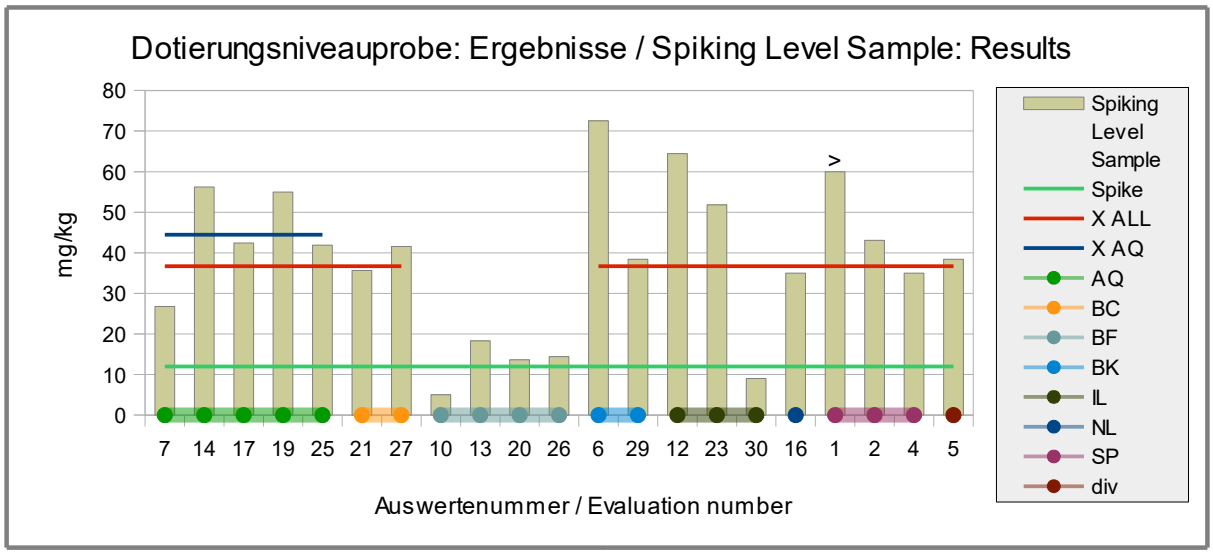


Abb./Fig. 15: ELISA Results Walnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method AQ
round symbols = Applied methods (see legend)

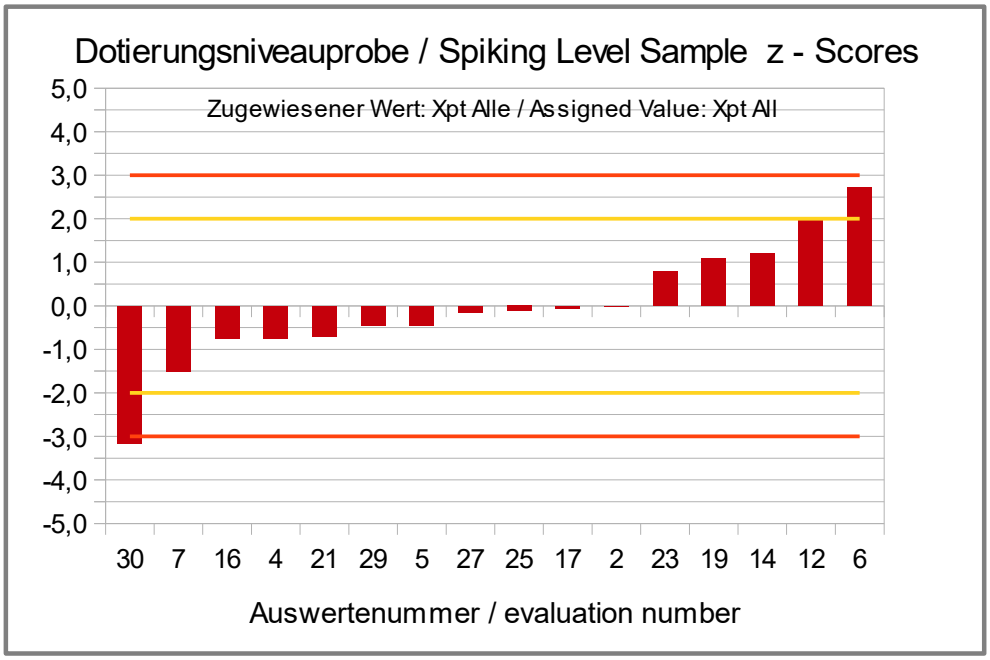


Abb./Fig. 16:
z-Scores (ELISA Results Walnut)
Assigned value robust mean of all results

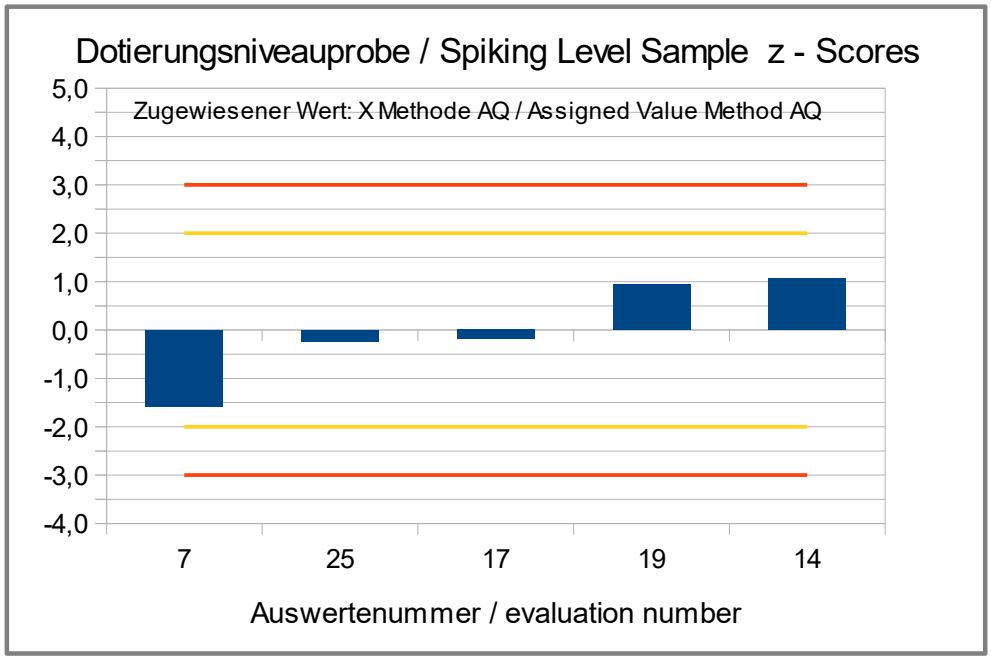


Abb./Fig. 17:
z-Scores (ELISA Results Walnut)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
7	26,8	224	5,0	<2,0			AQ	
14	56,2	470	15	10,6	**52**	-1,9	AQ	
17	42,4	355	10	8,50	42	-2,3	AQ	
19	55,0	460	14	9,00	44	-2,2	AQ	
25	41,9	350	10	3,80	19	-3,3	AQ	
21	35,7	298	7,9	4,25	21	-3,2	BC	
27	41,5	347	9,9	5,00	24	-3,0	BC	
10	5,00	42	-2,3	9,50	47	-2,1	BF	
13	18,3	153	2,1	9,03	44	-2,2	BF	
20	13,6	**114**	0,55	10,6	**52**	-1,9	BF	
26	14,4	**120**	0,82	11,6	**57**	-1,7	BF	
6	72,5	607	20	17,9	**87**	-0,50	BK	
29	38,4	321	8,8	15,8	**77**	-0,91	BK	
12	64,4	539	18	5,68	28	-2,9	IL	
23	51,8	433	13	6,10	30	-2,8	IL	
30	9,00	**75**	-1,0	3,20	16	-3,4	IL	
16	35,0	293	7,7	9,60	47	-2,1	NL	
1	>60			9,00	44	-2,2	SP	
2	43,1	360	10	8,40	41	-2,4	SP	
4	35,0	293	7,7	7,50	37	-2,5	SP	
5	38,4	321	8,8	7,70	38	-2,5	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	3	Number in RA	5
Percent in RA	15	Percent in RA	25

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
SP = SensiSpec ELISA Kit, Eurofins
div = not indicated / other method

Comments:

For the spiking level sample 15% (3) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A 25% (5) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.2.2 PCR Results: Walnut

Qualitative evaluation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
3	positive		negative		2/2 (100%)	SFA	
5	positive		negative		2/2 (100%)	SFA	
11	positive		negative		2/2 (100%)	SFA	
15	positive		negative		2/2 (100%)	SFA	
27	positive		negative		2/2 (100%)	SFA	
8	negative		negative		1/2 (50%)	SFA-4p	no positive sample identified
13	positive		negative		2/2 (100%)	SFA-ID	
4	positive		negative		2/2 (100%)	div	
16	negative		negative		1/2 (50%)	div	no positive sample identified

	Sample A		Sample B	
Number positive	7		0	
Number negative	2		9	
Percent positive	78		0	
Percent negative	22		100	
Consensus value	positive		negative	

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
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 A.

One negative result for sample A was obtained with the method SFA-4p (Sure Food Allergen 4plex, R-Biopharm / Congen) and a method not specifically stated, each.

Qualitative evaluation PCR: Spiking Level Sample

Evaluation number	Walnut	Walnut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
3	positive			SFA	
5	positive			SFA	
11	positive			SFA	
15	positive			SFA	
27	positive			SFA	
8	positive			SFA-4p	
13	positive			SFA-ID	
4	positive			div	
16	positive			div	

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative evaluation PCR: Sample A and Spiking Level Sample

No quantitative results were submitted by the participants.

4.3 Proficiency Test Egg

4.3.1 ELISA Results: Egg (as whole egg powder)

Qualitative evaluation 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		
19	negative	<LOQ	negative	<LOQ	1/2 (50%)	AQ-P	no positive sample identified
25	negative	<1,54	negative	<1,54	1/2 (50%)	AQ-P	no positive sample identified / result converted °
30	positive	<1,54	negative	<1,54	2/2 (100%)	IL	result converted °
2	positive	4,14	negative	<0,66	2/2 (100%)	MI-II	result converted °
4	positive	4,05	negative	<0,66	2/2 (100%)	MI-II	result converted °
6	positive	3,35	negative	<0,66	2/2 (100%)	MI-II	result converted °
10	positive	8,85	negative	<3,85	2/2 (100%)	MI-II	result converted °
13	positive	6,92	negative	<3,85	2/2 (100%)	MI-II	result converted °
26	positive	6,18	negative	<0,66	2/2 (100%)	MI-II	result converted °
16	positive	3,11	negative	<0,13	2/2 (100%)	RS	
21	positive	3,48	negative	<0.25	2/2 (100%)	RS	
24	positive	4,60	negative	<0,25	2/2 (100%)	RS	
27a	positive	3,05	negative	<0.25	2/2 (100%)	RS	
28	positive	3,30	negative	<0,5	2/2 (100%)	RS	
7	negative	<0.5	negative	<0.5	1/2 (50%)	RS-F	no positive sample identified
8	positive	0,290	negative	0,0300	2/2 (100%)	RS-F	result converted °
9	positive	<0,5	negative		2/2 (100%)	RS-F	
11	negative	<1,07	negative	<1,07	1/2 (50%)	RS-F	no positive sample identified / result converted °
12	negative		negative		1/2 (50%)	RS-F	no positive sample identified
18	negative	<1,15	negative	<1,15	1/2 (50%)	RS-F	no positive sample identified / result converted °
20	negative	<0,5	negative	<0,5	1/2 (50%)	RS-F	no positive sample identified
23	negative	<0,5	negative	<0,5	1/2 (50%)	RS-F	no positive sample identified
27b	positive	0,370	negative	<0.5	2/2 (100%)	RS-F	
22	positive	<1	negative	<0,5	2/2 (100%)	VT	
29	negative	0	negative	0	1/2 (50%)	VT	no positive sample identified
5	negative	<0,5	negative	<0,5	1/2 (50%)	div	no positive sample identified

° calculation see p. 19

	Sample A		Sample B	
Number positive	16		0	
Number negative	10		26	
Percent positive	62		0	
Percent negative	38		100	
Consensus value	none		negative	

Methods:

AQ-P = AgraQuant Plus, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:
For sample A, no consensus value with at least 75% concurring results was obtained for the various ELISA methods. While 100% positive results were obtained for the results of methods MI-II (Morinaga) and RS (R-Biopharm), the results for all other methods were at or below the respective limit of quantification (see documentation).
The negative consensus value of sample B is in qualitative agreement with the spiking of sample A.

Quantitative evaluation of ELISA-results: Sample A

Evaluation number	Whole egg powder	z-Score Xpt_{ALL}	z-Score Xpt_{MI-II}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]					
19	<LOQ				AQ-P	
25	<1,54				AQ-P	result converted °
30	<1,54				IL	result converted °
2	4,14	-0,30	-1,0		MI-II	result converted °
4	4,05	-0,38	-1,1		MI-II	result converted °
6	3,35	-1,0	-1,6		MI-II	result converted °
10	8,85	3,9	2,3		MI-II	result converted °
13	6,92	2,2	1,0		MI-II	result converted °
26	6,18	1,5	0,43		MI-II	result converted °
16	3,11	-1,2		-0,39	RS	
21	3,48	-0,89		0,04	RS	
24	4,60	0,11		1,3	RS	
27a	3,05	-1,3		-0,46	RS	
28	3,30	-1,1		-0,17	RS	
7	<0.5				RS-F	
8	0,290				RS-F	Outlier / result converted °
9	<0,5				RS-F	
11	<1,07				RS-F	result converted °
12					RS-F	
18	<1,15				RS-F	result converted °
20	<0,5				RS-F	
23	<0,5				RS-F	
27b	0,370				RS-F	Outlier / result converted °
22	<1				VT	
29	0				VT	
5	<0,5				div	

° calculation see p. 19

Methods:
AQ-P = AgraQuant Plus, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

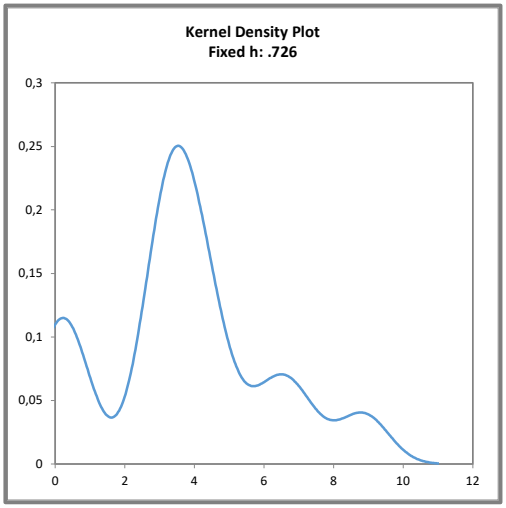


Abb. / Fig. 18:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an approximately symmetrical distribution of the results of the main peak with a secondary peak at approx. 0,2 mg/kg (outliers with methods RS-F and VT) and 2 further secondary peaks at around 6,5 mg/kg and 9 mg/kg ascribable to method MI-II.

Characteristics: Quantitative evaluation ELISA Egg (as whole egg powder)

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method MI-II** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ MI-II}}$	$X_{pt_{METHOD\ RS}}$
Number of results	11°	6	5
Number of outliers	2	0	0
Mean	4,64	5,58	3,51
Median	4,05	5,16	3,30
Robust Mean *(Xpt)*	**4,48**	**5,58**	**3,44**
Robust standard deviation (S*)	**1,74**	**2,39**	**0,561**
Target range:			
Target standard deviation σ_{pt}	**1,12**	**1,40**	**0,861**
lower limit of target range	**2,24**	**2,79**	**1,72**
upper limit of target range	**6,72**	**8,37**	**5,16**
Quotient S/σ_{pt}*	*1,6*	*1,7*	*0,65*
Standard uncertainty $U_{(Xpt)}$	*0,656*	*1,22*	*0,313*
Results in the target range	*9*	*5*	*5*
Percent in the target range	*82*	*83*	*100*

° *without results 8 and 27b (excluded in advance)*

Methods:
MI-II = Morinaga Institute ELISA Kit II
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The qualitative results as well as the kernel density estimation showed method-dependent differences. Quantitative results above the limits of quantification of the methods were only available for methods MI-II and RS. The results were evaluated together and separately according to methods.

The evaluations of the results of all methods (MI-II and RS) as well as of method MI-II and method RS showed a normal or low variability of the results. The quotient S/σpt was below 2.0 or below 1.0 in each case.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 22%, 27% and 17% of the spiking level of egg to sample A and thus below the range of the recommendations for the applied methods (s. 3.4.3 and p. 60 "Recovery rates with z-Scores ELISA for Egg").

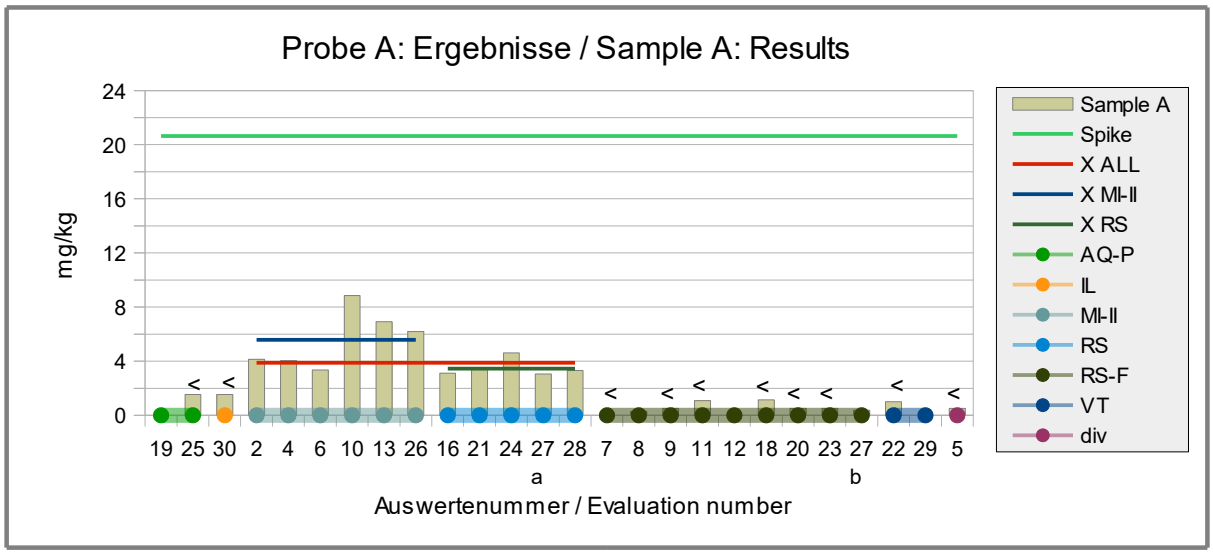


Abb./Fig. 19: ELISA Results Egg (as whole egg powder)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method MI-II
dark green line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

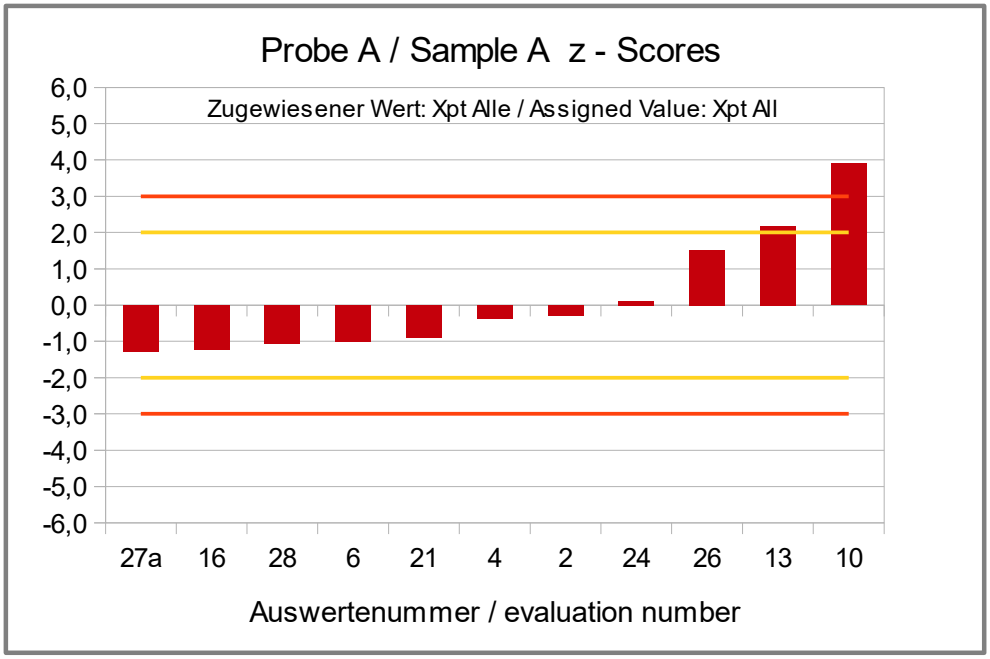


Abb./Fig. 20:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of all results

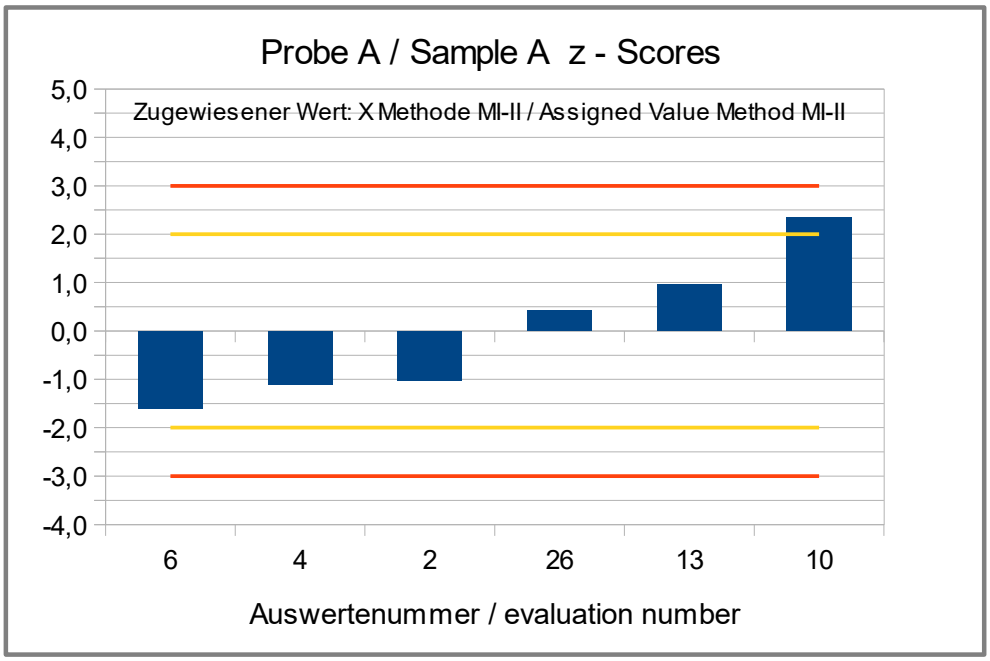


Abb./Fig. 21:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of method MI-II (Morinaga Institute ELISA Kit II)

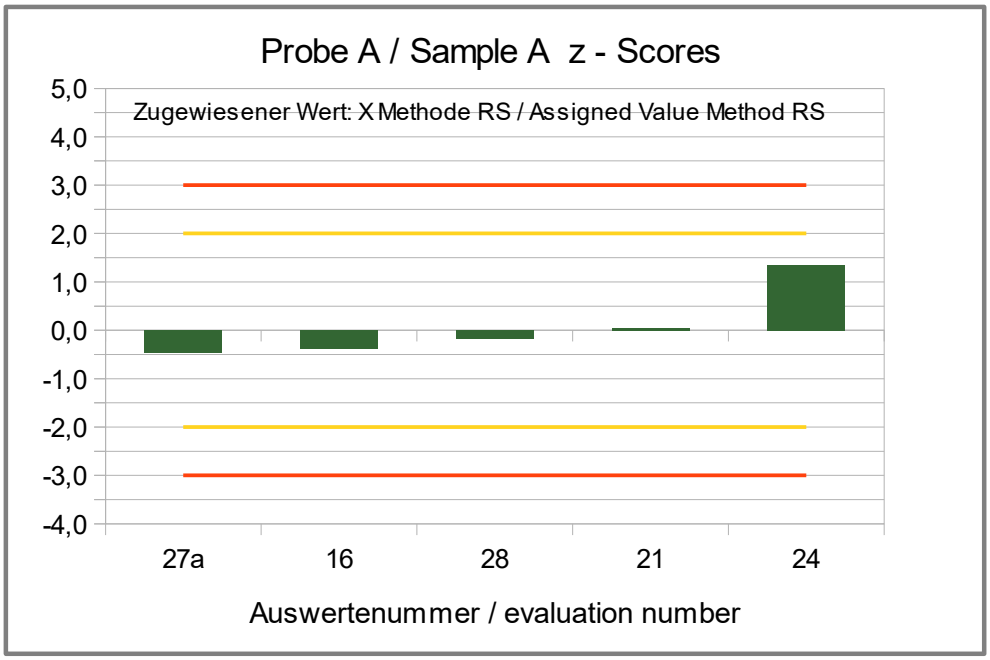


Abb./Fig. 22:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of method RS (R-Biopharm, Ridascreen®)

Quantitative evaluation of results: Spiking Level Sample

Evaluation number	Whole egg powder	z-Score Xpt_{ALL}	z-Score Xpt_{MI-II}	z-Score Xpt_{RS}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]						
19	20,0	1,8				AQ-P	
25	15,4	0,44				AQ-P	result converted °
30	8,88	-1,4				IL	result converted °
2	13,2	-0,18	0,22			MI-II	result converted °
4	12,8	-0,31	0,08			MI-II	result converted °
6	10,9	-0,85	-0,52			MI-II	result converted °
10	11,9	-0,56	-0,20			MI-II	result converted °
13	19,5	1,6	2,2			MI-II	result converted °
26	11,1	-0,80	-0,46			MI-II	result converted °
16	13,9	0,01		0,10		RS	
21	14,6	0,20		0,29		RS	
24	15,7	0,52		0,62		RS	
27a	12,7	-0,33		-0,25		RS	
28	11,0	-0,83		-0,76		RS	
7	10,0	-1,1			-0,86	RS-F	
8	12,5	-0,40			-0,08	RS-F	result converted °
9	10,2	-1,1			-0,79	RS-F	
11	22,6	2,5			3,1	RS-F	result converted °
12	10,8	-0,89			-0,61	RS-F	
18	13,1	-0,21			0,13	RS-F	result converted °
20	7,40	-1,9			-1,7	RS-F	
23	14,2	0,10			0,47	RS-F	
27b	17,3	1,0			1,4	RS-F	
22	24,0	2,9				VT	
29	80,8	19				VT	result converted °
5	12,7	-0,33				div	

° calculation see p. 19

Methods:

AQ-P = AgraQuant Plus, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

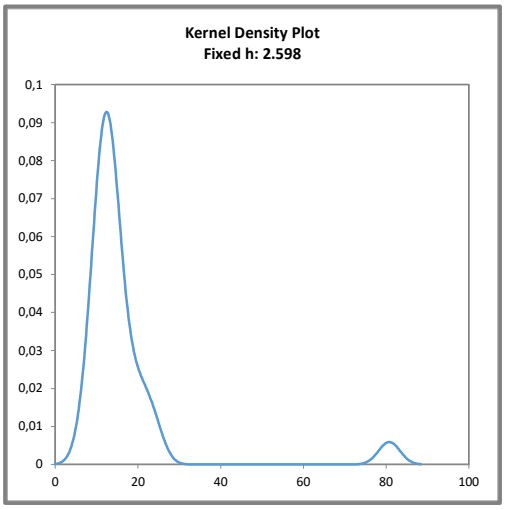


Abb. / Fig. 23:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimate shows an approximately symmetrical distribution with a slight shoulder at > 20 mg/kg and a secondary peak at approx. 80 mg/kg, which is due to a high single value (method VT).

Characteristics: Quantitative evaluation ELISA Egg (as whole egg powder)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method MI-II** *[mg/kg]*	**Method RS** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ MI-II}}$	$X_{pt_{METHOD\ RS}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	26	6	5	9
Number of outliers	–	–	0	0
Mean	16,4	13,2	13,6	13,1
Median	13,0	12,4	13,9	12,5
Robust Mean (X_{pt})	**13,9**	**12,5**	**13,6**	**12,7**
Robust standard deviation (S*)	**4,20**	**1,86**	**2,02**	**4,18**
Target range:				
Target standard deviation σ_{pt}	**3,46**	**3,14**	**3,39**	**3,18**
lower limit of target range	**6,93**	**6,27**	**6,78**	**6,36**
upper limit of target range	**20,8**	**18,8**	**20,3**	**19,1**
Quotient S/σ_{pt}*	*1,2*	*0,59*	*0,60*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*1,03*	*0,950*	*1,13*	*1,74*
Results in the target range	*23*	*5*	*5*	*8*
Percent in the target range	*88*	*83*	*100*	*89*

Methods:
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (one high single value by method VT).

The distribution of the results for all methods as well as for methods MI-II, RS and RS-F showed normal or low variability in each case. The quotients S/σpt were below 2,0 and below 1,0, respectively. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 107%, 96%, 104% and 97% of the spiking level of egg to the spiking level sample within the range of the recommendations for the applied methods (s. 3.4.3 and p. 60 "Recovery rates with z-scores ELISA for Egg").

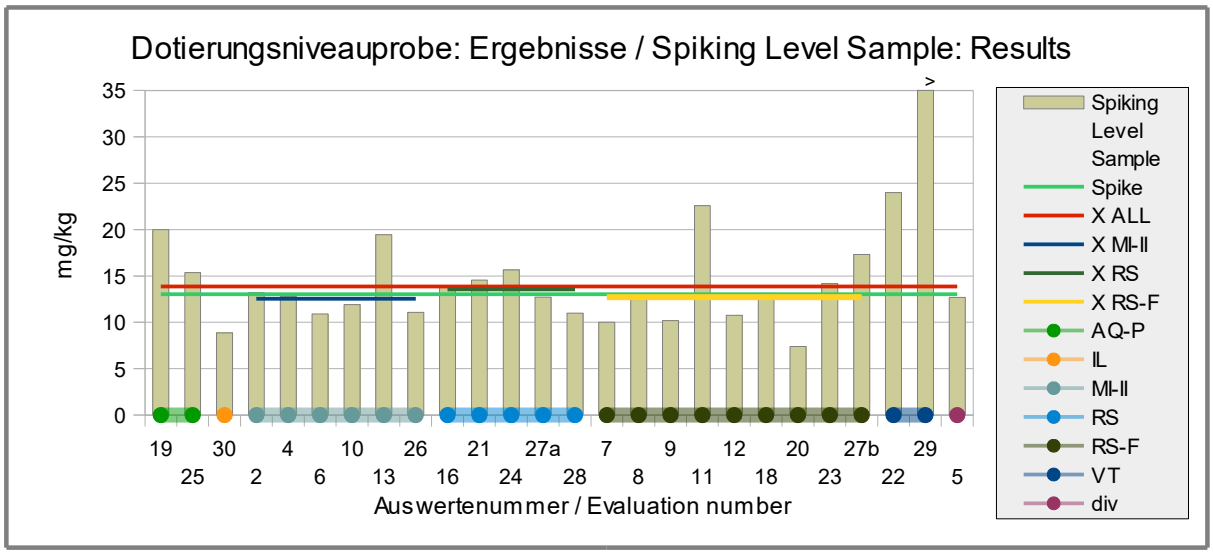


Abb./Fig. 24: ELISA Results Egg (as whole egg powder)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method MI-II
dark green line = Assigned value robust mean method RS
yellow line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

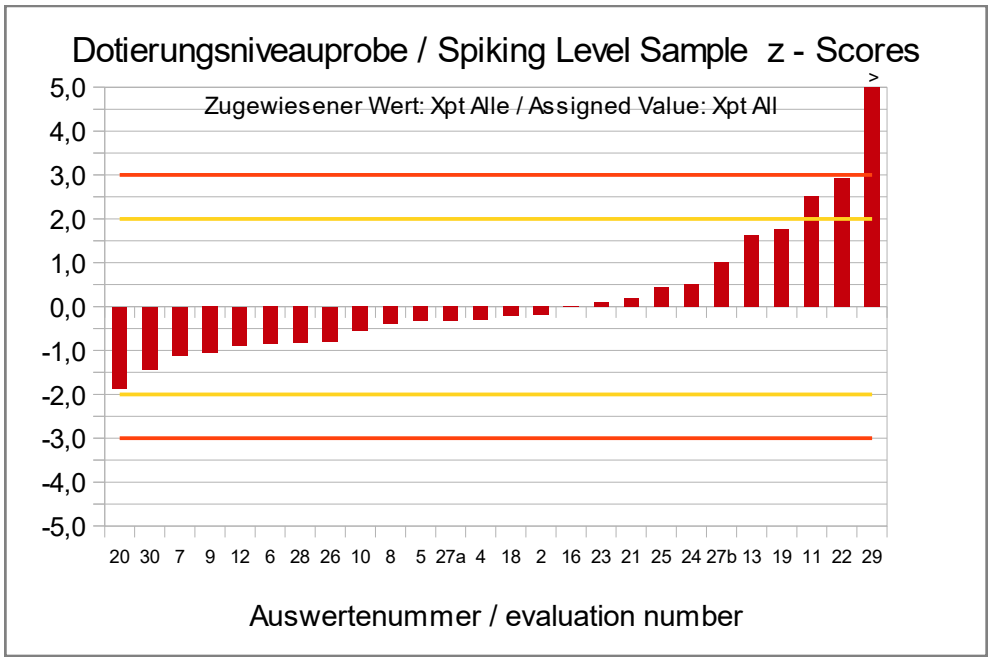


Abb./Fig. 25:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of all results

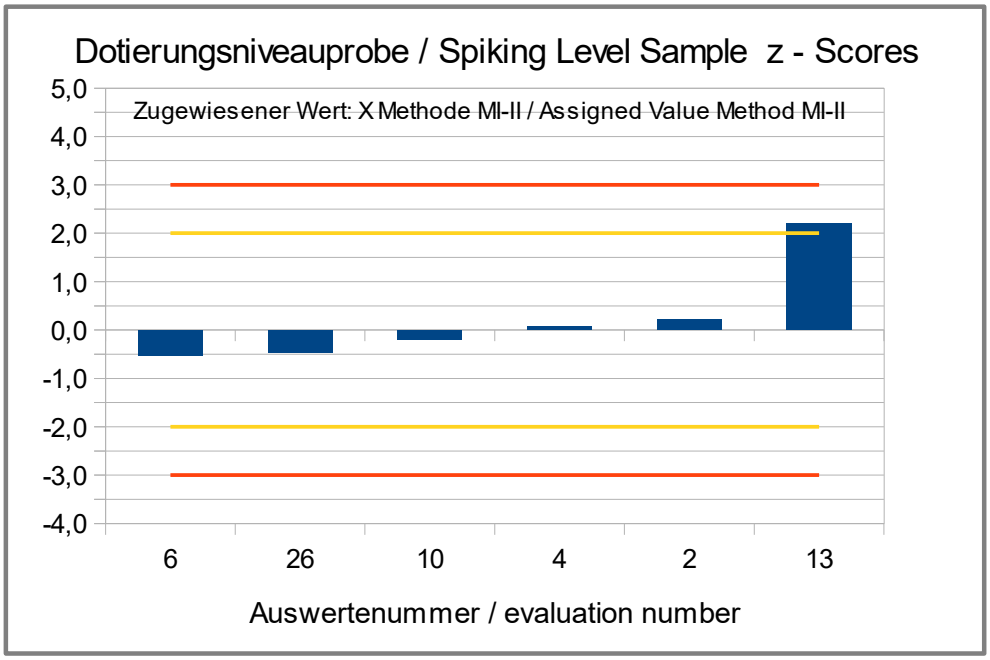


Abb./Fig. 26:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of method MI-II (Morinaga Institute ELISA Kit II)

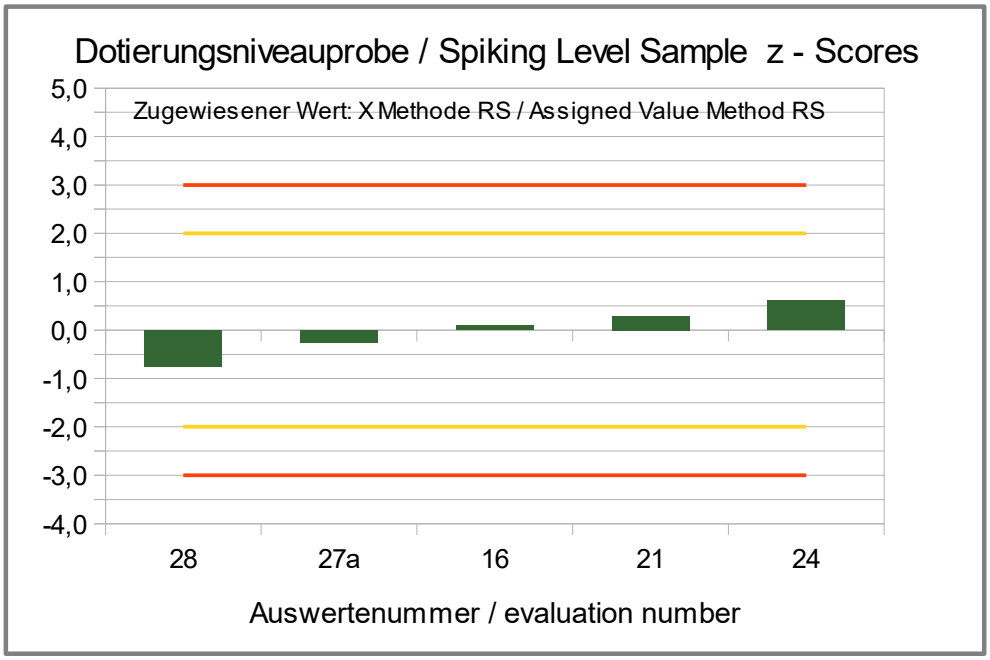


Abb./Fig. 27:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of method RS (Ridascreen®, R-Biopharm)

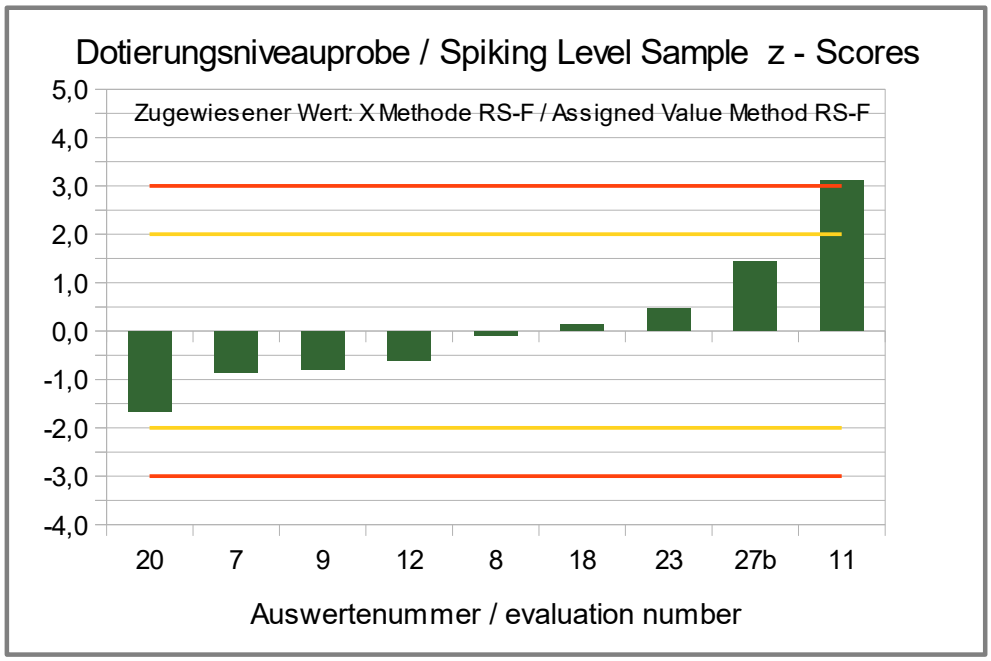


Abb./Fig. 28:
z-Scores ELISA Results Egg (as whole egg powder)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
19	20,0	153	2,1	<LOQ			AQ-P	
25	15,4	**118**	0,72	<1,54			AQ-P	result converted °
30	8,88	**68**	-1,3	<1,54			IL	result converted °
2	13,2	**101**	0,06	4,14	20	-3,2	MI-II	result converted °
4	12,8	**98**	-0,07	4,05	20	-3,2	MI-II	result converted °
6	10,9	**84**	-0,65	3,35	16	-3,4	MI-II	result converted °
10	11,9	**91**	-0,34	8,85	43	-2,3	MI-II	result converted °
13	19,5	**149**	2,0	6,92	34	-2,7	MI-II	result converted °
26	11,1	**85**	-0,60	6,18	30	-2,8	MI-II	result converted °
16	13,9	**107**	0,27	3,11	15	-3,4	RS	
21	14,6	**112**	0,47	3,48	17	-3,3	RS	
24	15,7	**120**	0,80	4,60	22	-3,1	RS	
27a	12,7	**98**	-0,10	3,05	15	-3,4	RS	
28	11,0	**84**	-0,62	3,30	16	-3,4	RS	
7	10,0	**77**	-0,93	<0.5			RS-F	
8	12,5	**96**	-0,17	0,290	1,4	-3,9	RS-F	result converted °
9	10,2	**78**	-0,87	<0,5			RS-F	
11	22,6	173	2,9	<1,07			RS-F	result converted °
12	10,8	**83**	-0,69				RS-F	
18	13,1	**101**	0,03	<1,15			RS-F	result converted °
20	7,40	**57**	-1,7	<0,5			RS-F	
23	14,2	**109**	0,36	<0,5			RS-F	
27b	17,3	**133**	1,3	0,370	1,8	-3,9	RS-F	
22	24,0	184	3,4	<1			VT	
29	80,8	620	21	0			VT	result converted °
5	12,7	**97**	-0,10	<0,5			div	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	22	Number in RA	0
Percent in RA	85	Percent in RA	0

* Recovery rate 100% relative size: Whole egg powder, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ-P = AgraQuant Plus, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:

85% (22) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150% with the spiking level sample. For the processed, spiked food matrix sample A, none of the recovery rates were in this acceptance range, whereas the recovery rates for methods MI-II and RS were in the range ≥ 15%.
The related z-scores are based on the target standard deviation of 25%.

4.3.2 PCR Results: Egg

Evaluation PCR: Sample A and Spiking Level Sample

No results were submitted by the participants.

4.4 Participant z-Scores: overview table

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

Evaluation number	ELISA Hazelnut: **Xpt** (div. Methods)		ELISA Hazelnut: **Xpt** (Method: RS-F)		ELISA Walnut: **Xpt** (div. Methods)		ELISA Walnut: **Xpt** (Method: AQ)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1	1,8	0,23			0,28			
2	-0,63	0,61			0,00	0,69		
3								
4	0,32	-1,8			-0,43	-0,19		
5		0,19	-1,2	-0,40	-0,34	0,18		
6		1,8	-0,59	0,98	4,5	3,9		
7		1,1	0,99	0,36		-1,1		-1,6
8								
9		0,80	0,26	0,13				
10		-3,0	-0,92	-3,2	0,52			
11								
12	-0,60	-1,0			-1,3	3,0		
13	0,01	2,9			0,30			
14	0,21	-1,5			1,0	2,1		1,1
15								
16		1,6	0,66	0,78	0,57	-0,19		
17		-0,70	-0,33	-1,2	0,05	0,62		-0,19
18		1,3	0,27	0,59				
19	-0,76	-0,16			0,28	2,0		0,95
20		-0,23	-0,23	-0,77	1,0			
21	0,03	0,01			-2,0	-0,11		
22	0,40	-1,1						
23	0,01	-0,50			-1,1	1,6		
24		1,1	0,48	0,34				
25	0,21	-0,39			-2,2	0,56		-0,23
26	-0,12	0,00			1,5			
27		1,2	0,75	0,48	-1,6	0,53		
28		0,61	-0,19	-0,04				
29	0,92	-1,2			3,5	0,18		
30	-1,3	-3,0			-2,5	-3,0		

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

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

Evaluation number	ELISA Egg: Xpt (div. Methods)		ELISA Egg: Xpt (Method: MI-II)		ELISA Egg: Xpt (Method: RS)		ELISA Egg: Xpt (Method: RS-F)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1								
2	-0,30	-0,18	-1,0	0,22				
3								
4	-0,38	-0,31	-1,1	0,08				
5		-0,33						
6	-1,0	-0,85	-1,6	-0,52				
7		-1,1						-0,86
8		-0,40						-0,08
9		-1,1						-0,79
10	3,9	-0,56	2,3	-0,20				
11		2,5						3,1
12		-0,89						-0,61
13	2,2	1,6	1,0	2,2				
14								
15								
16	-1,2	0,01			-0,39	0,10		
17								
18		-0,21						0,13
19		1,8						
20		-1,9						-1,7
21	-0,89	0,20			0,04	0,29		
22		2,9						
23		0,10						0,47
24	0,11	0,52			1,3	0,62		
25		0,44						
26	1,5	-0,80	0,43	-0,46				
27a	-1,3	-0,33			-0,46	-0,25		
27b		1,0						1,4
28	-1,1	-0,83			-0,17	-0,76		
29		19						
30		-1,4						

Methods: MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F = Ridascreen® Fast, R-Biopharm

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

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

Evaluation number	ELISA Hazelnut		ELISA Walnut		ELISA Egg		PCR Hazelnut	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1	-2,2	-0,36	-2,2					
2	-3,0	-0,03	-2,4	10	-3,2	0,06		
3								
4	-2,7	-2,1	-2,5	7,7	-3,2	-0,07		
5	-2,3	-0,40	-2,5	8,8		-0,10		
6	-2,0	1,0	-0,50	20	-3,4	-0,65		
7	-1,1	0,36		5,0		-0,93		
8					-3,9	-0,17		
9	-1,5	0,13				-0,87		
10	-2,2	-3,2	-2,1	-2,3	-2,3	-0,34		
11						2,9		
12	-2,9	-1,4	-2,9	18		-0,69		
13	-2,7	1,9	-2,2	2,1	-2,7	2,0		
14	-2,7	-1,8	-1,9	15				
15								
16	-1,3	0,78	-2,1	7,7	-3,4	0,27		
17	-1,9	-1,2	-2,3	10				
18	-1,5	0,60				0,03		
19	-3,0	-0,69	-2,2	14		2,1		
20	-1,8	-0,76	-1,9	0,55		-1,7		
21	-2,7	-0,55	-3,2	7,9	-3,3	0,47		
22	-2,6	-1,5				3,4		
23	-2,7	-1,0	-2,8	13		0,36		
24	-1,4	0,35			-3,1	0,80		
25	-2,7	-0,89	-3,3	10		0,72		
26	-2,8	-0,56	-1,7	0,82	-2,8	-0,60		
27 / 27a	-1,2	0,49	-3,0	9,9	-3,4	-0,10	-3,4	0,01
27b					-3,9	1,3		
28	-1,8	-0,03			-3,4	-0,62		
29	-2,5	-1,6	-0,91	8,8		21		
30	-3,2	-3,1	-3,4	-1,0		-1,3		

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Hazelnut

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
AQ	19	21.10.21	positive	5	negative	<LOQ	positive	10	0,3	1	50	Hazelnut	AgraQuant ELISA Hazelnut COKAL0348, RomerLabs
AQ	21	06.10.21	-	6,231	-	<1	-	10,43		1	39,15	Hazelnut	AgraQuant ELISA Hazelnut COKAL0348, RomerLabs
AQ	25	19.10.21	positive	6,5	negative	<1	positive	9,4		1		Hazelnut	AgraQuant ELISA Hazelnut COKAL0348, RomerLabs
BF	13		positive	6,2	negative	<1	positive	17,9		1		Hazelnut	MonoTrace Hazelnut ELISA kit, BioFront Technologies
IL	12	01.10.21	positive	5,26	negative		positive	7,81		1	11	Hazelnut	Immunolab Hazelnut ELISA
IL	23	21.09.21	positive	6,2	negative	<1	positive	9,1		1		Hazelnut	Immunolab Hazelnut ELISA
IL	30		positive	4,1	negative	<1	positive	2,7				Hazelnut	Immunolab Hazelnut ELISA
MI-II	4		positive	0,94	negative	<0,16	positive	0,82	0,16	0,16		Hazelnut protein	Morinaga Hazelnut ELISA Kit II
RS-F	5	07.09.21	-	8,2	-	<2,5	-	10,9	0,19	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	6	16.09.21	positive	9,86	negative	<2.5	positive	15,08	N/A	2,5		Please select!	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	7	06.09.21	positive	14,4	negative	<2.5	positive	13,2	1,5	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	9	13.09./14.09./ 27.09.	positive	12,3	negative		positive	12,5	0,19	2,5	50	Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	10	17.09.21	positive	8,9	negative	<2,5	negative	2,5		2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	16	09.09.	positive	13,45	negative	<0,2	positive	14,47	0,2	2,5	20	Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	17	18.10.21	positive	10,6	negative		positive	8,6		2,5	40	Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	18	13.09.21	positive	12,33	negative	<2.5	positive	13,92				Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	20	31/09	positive	10,9	negative	<2,5	positive	9,8	-	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	24	24.09.21	positive	12,93	negative	<2,5	positive	13,15	0,19	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	27	13.09.21	positive	13,72	negative	<2.5	positive	13,58	2,5	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	28	01.10.21	positive	11	negative	<2,5	positive	12		2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
SP	1	07.09.21	positive	9	negative	0	positive	11	0.07	1		Hazelnut	Eurofins SensiSpec Hazelnut ELISA Kit
SP	2	09.09.21	positive	5,2	negative	<1.0	positive	12		1		Hazelnut	Eurofins SensiSpec Hazelnut ELISA Kit
VT	14	20.09.21	positive	6,5	negative	< 2,50	positive	6,55	0,85	2,5	45	Hazelnut	Veratox Hazelnut, Neogen
VT	22	06.10.21	positive	6,8	negative	<2,5	positive	7,5	1	2,5		Hazelnut	Veratox Hazelnut, Neogen
VT	26	22.10.21	pos	6	neg	<2.5	pos	10,4		2,5		hazelnut	Neogen Veratox
VT	29	10.09.21	-	7.6	-	0	-	7.4	1	3	26.6	Hazelnut	Veratox Hazelnut, Neogen

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. extraction solution / time / temperature	yes/no	
AQ	19			yes	
AQ	21			Yes	
AQ	25				
BF	13			yes	The suppliers who manufacture ELISA kit, used for the detection of the hazelnut allergen, alleges that cross-reactivity to walnut (1,08%).
IL	12			yes	
IL	23			yes	
IL	30				
MI-II	4	detects hazelnut proteins	according to manufacturer information	yes	
RS-F	5	polyclonal		yes	
RS-F	6		as the insert	yes	recovery in sample B = 55 %
RS-F	7			yes	
RS-F	9			yes	
RS-F	10			yes	
RS-F	16	Hazelnut proteins	Allergen extraction buffer with skimmed milk powder and extractor egg, 10 min / 60 ° C	yes	
RS-F	17			yes	
RS-F	18				
RS-F	20		According to instructions	yes	
RS-F	24	Hazelnut proteins	As Per Kit Instructions	yes	
RS-F	27	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	28			yes	
SP	1				
SP	2			Yes	
VT	14			yes	
VT	22			no	
VT	26				
VT	29		PBS/15 min/60C	yes	

5.1.2 ELISA: Walnut

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
AQ	7	16.09.21	negative	<2.0	negative	<2.0	positive	26,8	0,35	2		Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	14	16.09.21	positive	10,59	negative	< 6,00	positive	56,2	0,35	6	18	Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	17	18.10.21	positive	8,5	negative		positive	42,4		2	40	Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	19	21.10.21	positive	9	negative	<LOQ	positive	55	0,35	2	50	Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	25	22.10.21	positive	3,8	negative	<2	positive	41,9		2		Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
BC	21	19.10.21	-	4,25	-	<2	-	35,67		2	26,28	Walnut	BioCheck ELISA Walnut-Check
BC	27	13.09.21	positive	5	negative	<2	positive	41,54	2	2		Walnut	BioCheck ELISA Walnut-Check
BF	10	14.09.21	positive	9,5	negative	<2	positive	5		2		Walnut	
BF	13		positive	9,03	negative	<1	positive	18,3		1		Walnut	MonoTrace Walnut ELISA kit, BioFront Technologies
BF	20	03.10.21	positive	10,6	negative	<1,0	positive	13,6	-	1		Hazelnut	MonoTrace Walnut ELISA kit, BioFront Technologies
BF	26	22.10.21	pos	11,6	neg	<2.0	pos	14,4		2		walnut	BioFront
BK	6	17.09.21	positive	17,87	negative	<2,4	positive	72,54	N/A	2,4		Walnut	BioKits Walnut Assay Kit, Neogen
BK	29	10.09.21	-	15.8	-	0	-	38.4	2	4	24.9	Walnut	BioKits Walnut Assay Kit, Neogen
IL	12	14.10.21	positive	5,68	negative		positive	64,44		2		Walnut	Immunolab Walnut ELISA
IL	23	21.09.21	positive	6,1	negative	<2	positive	51,8		2		Walnut	Immunolab Walnut ELISA
IL	30		positive	3,2	negative	<2	positive	9				Walnut	Immunolab Walnut ELISA
NL	16	14.09.	positive	9,6	negative	<0,6	positive	35	0,6	2	20	Walnut	nutriLinia® Walnut-ELISA
SP	1	19.08.22	positive	9	negative	0	positive	>60	0.4	2		Walnut	Eurofins SensiSpec Walnut ELISA Kit
SP	2	09.09.21	positive	8,4	negative	<2.0	positive	43,1		2		Walnut	Eurofins SensiSpec Walnut ELISA Kit
SP	4		positive	7,5	negative	<2	positive	35	1	2		Walnut	Eurofins SensiSpec Walnut ELISA Kit
div	5	08.09.21	-	7,7	-	<2,0	-	38,4	0,35	2		Walnut	Biosystem

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

Meth. Abbr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	7			yes	
AQ	14			yes	
AQ	17			no	
AQ	19			yes	
AQ	25				
BC	21			Yes	
BC	27	As Per Kit Instructions	As Per Kit Instructions	Yes	
BF	10			yes	BIOFRONT MONOTRACE WALNUT (WJ4-EK-96)
BF	13			yes	
BF	20		According to instructions	yes	
BF	26				
BK	6		as the insert	yes	recovery in sample B < 15%
BK	29		Extraction buffer/ 15min/ RT	yes	
IL	12			no	sample diluted tw ice
IL	23			yes	
IL	30				
NL	16	Walnut proteins	Allergen extraction buffer, / 15 min / 60 ° C	yes	
SP	1				
SP	2			Yes	
SP	4	detects w alnut proteins	According to manufacturer information	yes	
div	5	polyclonal		yes	

5.1.3 ELISA: Egg

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
AQ-P	19	21.10.21	negative	<LOQ	negative	<LOQ	positive	20	0,5	1	50	total	AgraQuant Plus ELISA Egg COKAL1848F, RomerLabs
AQ-P	25	18.10.21	negative	<0.4	negative	<0.4	positive	4		0,4		Egg white proteins, total	AgraQuant Plus ELISA Egg COKAL1848F, RomerLabs
IL	30		positive	<0,4	negative	<0,4	positive	2,31				Egg white proteins, total	Immunolab Egg white ELISA
MI-II	2	09.09.21	positive	1,94	negative	<0.31	positive	6,2		0,31		Whole egg protein	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)
MI-II	4		positive	1,9	negative	<0,31	positive	6	0,31	0,31		Whole egg protein	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)
MI-II	6	28.09.21	positive	1,57	negative	<0,31	positive	5,11	N/A	0,31		egg protein	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)
MI-II	10	13.09.21	positive	2,3	negative	<1	positive	3,1		1		Egg white proteins, total	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)
MI-II	13		positive	1,8	negative	<1	positive	5,06		1		Egg white proteins, total	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)
MI-II	26	04.09.21	pos	2,9	neg	<0.31	pos	5,2		0,31		whole egg protein	Morinaga Elisa Kit II
RS	16	15.09.	positive	3,11	negative	<0,13	positive	13,9	0,13	0,25	20	Whole egg powder	Ridascreen® Egg R6411, R-Biopharm
RS	21	08.10.21	-	3,48	-	<0.25	-	14,55		0,25	30,22	Whole egg powder	Ridascreen® Egg R6411, R-Biopharm
RS	24	21.09.21	positive	4,6	negative	<0,25	positive	15,65	0,13	0,25		Whole egg powder	Ridascreen® Egg R6411, R-Biopharm
RS	27a	13.09.21	positive	3,05	negative	<0.25	positive	12,72	0,25	0,25		Whole egg powder	Ridascreen® Egg R6411, R-Biopharm
RS	28	06.10.21	positive	3,3	negative	<0,5	positive	11		0,5		Whole egg powder	Ridascreen® Egg R6411, R-Biopharm
RS-F	7	02.09.21	negative	<0.5	negative	<0.5	positive	10	0,1	0,5		Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	8	06.10.21	positive	0,076	negative	0,009	positive	3,24	0,03	0,13		Egg white proteins, total	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	9	08.09./14.09./ 16.09./04.10.	positive	<0,5	negative		positive	10,2	0,1	0,5	50	Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	11	22.09.21	-	<0,5	-	<0,5	-	10,6		0,5		EGG PROTEIN	ELISA R6402 R-BIOPHARM
RS-F	12	06.10.21	negative		negative		positive	10,77		0,5	17	Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	18	14.09.21	negative	<0.3	negative	<0.3	positive	3,41				Egg white proteins, total	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	20	31/09	negative	<0,5	negative	<0,5	positive	7,4	-	0,5		Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	23	21.09.21	negative	<0,5	negative	<0,5	positive	14,2		0,5		Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	27b	13.09.21	positive	0,37	negative	<0.5	positive	17,31	0,5	0,5		Whole egg powder	Ridascreen® FAST Egg Protein R6402, R-Biopharm
VT	22	08.10.21	positive	<1	negative	<0,5	positive	24	0,5	1		Whole egg powder	Veratox Egg Allergen, Neogen
VT	29	10.09.21	-	0	-	0	-	21	1	2	28.6	Egg white proteins, total	Veratox Egg Allergen, Neogen
div	5	08.09.21	-	<0,5	-	<0,5	-	12,7	0,1	0,5		Whole egg powder	Selection Egg-Kits:

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

Meth. Abbr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ-P	19			yes	
AQ-P	25				
IL	30				
MI-II	2			Yes	
MI-II	4	determines the egg white protein ovalbumin	According to manufacturer information	yes	
MI-II	6		as the insert	yes	recovery in sample B = 94 %
MI-II	10			yes	
MI-II	13			yes	
MI-II	26				
RS	16	Ovalbumin/ovomucoid	Allergen extraction buffer with additives1, skimmed milk powder and extractor egg, 10 min / 60 ° C	yes	
RS	21			Yes	
RS	24	egg white proteins	As Per Kit Instructions	yes	
RS	27a	As Per Kit Instructions	As Per Kit Instructions	Yes	Processed Egg Kit
RS	28			yes	
RS-F	7			yes	
RS-F	8	specifically egg white proteins ovalbumin and ovomucoid	Extraction solution 60 ° C		
RS-F	9			yes	
RS-F	11			YES	
RS-F	12			yes	
RS-F	18				
RS-F	20		According to instructions	yes	
RS-F	23			yes	
RS-F	27b	As Per Kit Instructions	As Per Kit Instructions	Yes	Result estimated (lower than LOD)
VT	22			no	
VT	29		PBS/15 min/60C	YES	
div	5	polyclonal		YES	Sample A positive below LOQ

5.1.4 PCR: Hazelnut

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
SFA	3		positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	07.09.21	positive		negative		positive		0,4			Hazelnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	13		positive		negative		positive		0,4			Hazelnut DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA	15		positive		NEGATIVE		POSITIVE		< 0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	27	15.09.21	positive	3,13	negative	<1	positive	12,13	1	1		Hazelnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	8	14.09.21	positive		NEGATIVE		POSITIVE		0,4			Hazelnut-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-4p	11	24.09.21	NEGATIVE		NEGATIVE		POSITIVE		0,4			Please select!	SureFood® ALLERGEN 4plex Peanut / Hazelnut / Walnut + IAC S3402

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target sequence / DNA	e.g. extraction / enzymes / clean-up / real-time PCR / gel electrophoresis / cycling	yes/no	
SFA	3				
SFA	5			YES	
SFA	13			yes	
SFA	15			yes	
SFA	27	As Per Kit Instructions	As Per Kit Instructions	No	
SFA-4p	8		SureFood® PREP Advanced Kit, protocol 1		
SFA-4p	11			NO	

5.1.5 PCR: Walnut

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
SFA	3		positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	07.09.21	positive		negative		positive		0,4			Walnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11	24.09.21	POSITIVE		NEGATIVE		POSITIVE		2			WALNUT	SUREFOOD ALLERGEN WALNUT S3607
SFA	15		positive		negative		positive		< 0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	27	15.09.21	positive		negative		positive		1	1		Walnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	8	14.09.21	negative		negative		positive		0,4			Walnut-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	13		positive		negative		positive		0,4			Walnut DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	4		positive		negative		positive		1			Walnut-DNA	internal method
div	16	18.10.	negative		negative		positive		10	100		Walnut-DNA	Brezna et al. Eur Food Res Technol, 2006: 223, 373-377.

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target sequence / DNA	e.g. extraction / enzymes / clean-up / real-time PCR / gel electrophoresis / cycling	yes/no	
SFA	3				
SFA	5			YES	
SFA	11			YES	
SFA	15			yes	
SFA	27	As Per Kit Instructions	As Per Kit Instructions	No	
SFA-4p	8		SureFood® PREP Advanced Kit, protocol 1		
SFA-ID	13			yes	
div	4		CTAB / Proteinase K / Rnase A / realtime PCR / 45 cycles	yes	
div	16	jug R2 7S	CTAB, Prot. K, Chloroform; Clean-up: FFS-Kit/Maxwell (Promega)	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL05 (2021) Sample A

Weight whole sample	2,80	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	27,1	mg/kg

Result of analysis

Sample	Weight (g)	Particle number	Particles [mg/kg]
1	5,02	57	22,7
2	4,99	67	26,9
3	5,02	58	23,1
4	4,99	71	28,5
5	5,05	74	29,3
6	4,98	63	25,3
7	4,99	69	27,7
8	5,00	72	28,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	66,4	Particle
Standard deviation	6,40	Particle
χ^2 (CHI-Quadrat)	4,32	
Probability	**74**	%
Recovery rate	98	%

Normal distribution		
Number of samples	8	
Mean	26,5	mg/kg
Standard deviation	2,56	mg/kg
rel. Standard deviaton	9,6	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,0**	
Recovery rate	98	%

Microtracer Homogeneity Test
DLA ptAL05 (2021) Spiking Level Sample

Weight whole sample	1,30	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,0	mg/kg

Result of analysis

Sample	Weight (g)	Particle number	Particles [mg/kg]
1	5,03	74	29,4
2	5,01	72	28,7
3	5,00	76	30,4
4	5,00	62	24,8
5	5,00	69	27,6
6	4,98	62	24,9
7	4,98	57	22,9
8	5,02	71	28,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	67,9	Particle
Standard deviation	6,58	Particle
χ^2 (CHI-Quadrat)	4,47	
Probability	**72**	%
Recovery rate	94	%

Normal distribution		
Number of samples	8	
Mean	27,1	mg/kg
Standard deviation	2,63	mg/kg
rel. Standard deviaton	9,7	%
Horwitz standard deviation	9,7	%
HorRat-value	**1,0**	
Recovery rate	94	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL05 - 2021***
PT name	***Allergens V: Hazelnut, Walnut and Egg in Pastry***
Sample matrix (processing)	***Samples A + B:*** *Cocoa biscuits (baked, 150°C) / Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt, other food additives and allergenic foods (hazelnut, walnut, whole egg powder; one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each + 1 Spiking Level Sample: 15 g*
Storage	*Samples A, B + Spiking Level Sample: room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Hazelnut (Hazelnut protein, DNA), Walnut (Walnut protein, DNA), Egg (Egg protein, DNA) Samples A + B: < 500 mg/kg Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest October 22nd 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		SPAIN
		SPAIN
		CANADA
		ITALY
		Germany
		Germany
		Germany
		FRANCE
		SPAIN
		Germany
		GREAT BRITAIN
		GREECE
		SPAIN
		SWITZERLAND
		SPAIN
		BELGIUM
		Germany
		Germany
		Germany
		SPAIN
		CANADA
		GREAT BRITAIN
		ITALY
		Germany
		AUSTRIA
		GREAT BRITAIN
		Germany
		SPAIN
		ITALY
		GREAT BRITAIN
		GREECE

[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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