Evaluation Report
proficiency test

DLA ptAL06 (2020)

Allergens VI:

Hazelnut and Pecan

in Chocolate

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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 As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s)
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.

Content

1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a mixture of commercially available dark chocolates (cocoa content: approx. 75-80%). The basic composition of both sample A and sample B was the same (see table 1). The basic mixture was homogenized by stirring at approx. 40°C.

Afterwards the **spiked sample B** was produced as follows:
The spiking material containing the allergenic ingredients hazelnut and pecan was added to an aliquot of the basic mixture and the mixture was homogenized at approx. 40°C. Subsequently, the basic mixture was again added in 4 additional steps and homogenized each until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

After homogenization the samples A and B were portioned to approx. 25 g into PE container and metallised PET film bags. The spiking level sample was portioned to approx. 15 g in metallized PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Probe A**	**Probe B**	**Dotierungs-niveauprobe**
Fine dark chocolates (85% and 70% cocoa content, 1:1 mixture) Ingredients: Cocoa mass, sugar, cocoa butter, emulsifier: lecithin (Soya), vanillin Nutrients per 100 g: Fat 46 g, Carbohydrates 27 g, Protein 10 g	100 g/100 g	99,7 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	–	–	99,7 g/100 g
Hazelnuts, roasted: ground, mixture (5 countries / Europe) – as Hazelnut* – *thereof 14,1% total protein***	–	 32,2 mg/kg 4,54 mg/kg	 36,2 mg/kg 5,10 mg/kg
Pecans, ground: – as Pecan* – *thereof 10,3% total protein***	–	 33,9 mg/kg 3,49 mg/kg	 35,2 mg/kg 3,63 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	–	<0,02 g/100 g	<0,02 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,30 for hazelnuts and pecans)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
Because stuck solid samples can not be analysed by the microtracer method, only the spiking level sample was measured. The microtracer analysis of the present PT showed a probability of 86%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value of 0,9. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis (exception: Morinaga Kit 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 B by all ELISA tests for hazelnut (Immunolab, Morinaga and AgraQuant) and pecan (house-method), respectively (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].

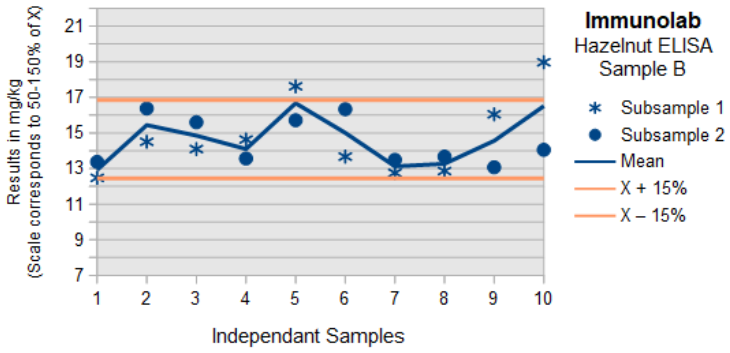
ELISA-Tests: Homogenität Haselnuss / Homogeneity Hazelnut

Immunolab Hazelnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut 14,6 ± 1,1 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	12,5	13,4	12,9
2	14,5	16,4	15,4
3	14,1	15,6	14,8
4	14,6	13,6	14,1
5	17,6	15,7	16,7
6	13,7	16,3	15,0
7	12,8	13,5	13,1
8	12,9	13,7	13,3
9	16,0	13,1	14,6
10	19,0	14,1	16,5

General average X	14,6	
SD of sample means Sx	1,33	9,1%
SD within-samples Sw	1,15	7,8%
SD between-samples Ss	1,05	7,2%

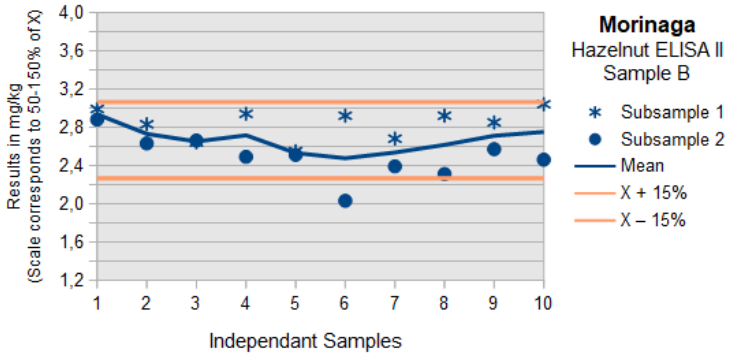


Morinaga Hazelnut ELISA II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut protein 2,66 ± 0,13 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	2,99	2,88	2,94
2	2,83	2,63	2,73
3	2,64	2,66	2,65
4	2,94	2,49	2,72
5	2,55	2,51	2,53
6	2,92	2,03	2,48
7	2,68	2,39	2,54
8	2,92	2,31	2,62
9	2,85	2,57	2,71
10	3,04	2,46	2,75

General average X	2,66	
SD of sample means Sx	0,13	5,0%
SD within-samples Sw	0,22	8,2%
SD between-samples Ss	< 0,13	< 5,0%

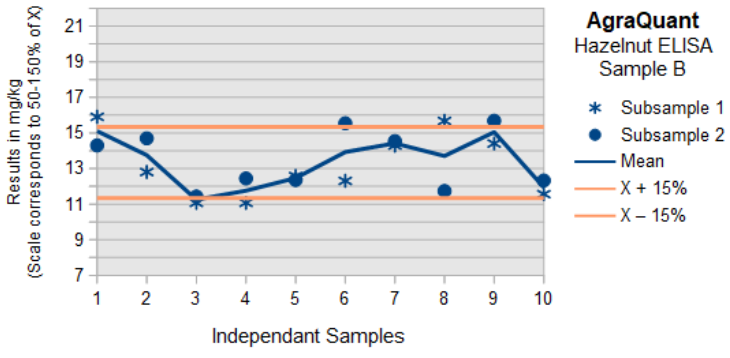


AgraQuant Hazelnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Hazelnut 13,3 ± 1,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	15,9	14,3	15,1
2	12,8	14,7	13,8
3	11,1	11,4	11,3
4	11,1	12,4	11,8
5	12,6	12,4	12,5
6	12,3	15,5	13,9
7	14,3	14,5	14,4
8	15,7	11,7	13,7
9	14,4	15,7	15,0
10	11,6	12,3	11,9

General average X	13,3	
SD of sample means Sx	1,39	10,4%
SD within-samples Sw	0,95	7,2%
SD between-samples Ss	1,21	9,1%



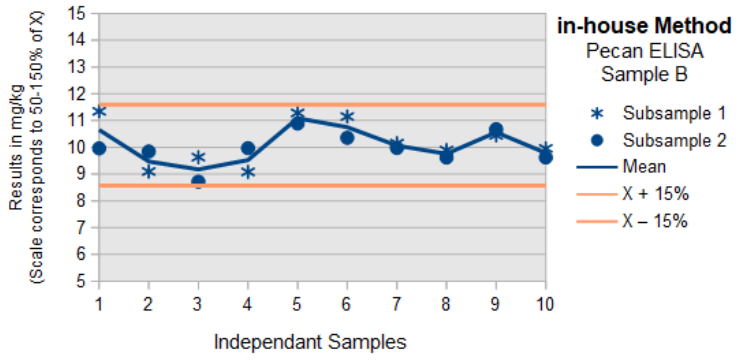
ELISA-Tests: Homogenität Pecannuss / Homogeneity Pecan

in-house Method Pecan ELISA

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

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	11,3	10,0	10,7
2	9,1	9,8	9,5
3	9,6	8,7	9,2
4	9,1	10,0	9,5
5	11,3	10,9	11,1
6	11,2	10,4	10,8
7	10,2	10,0	10,1
8	9,9	9,6	9,8
9	10,4	10,7	10,6
10	10,0	9,6	9,8

General average X	10,1	
SD of sample means Sx	0,64	6,4%
SD within-samples Sw	0,36	3,6%
SD between-samples Ss	0,59	5,9%



2.1.2 Stability

The food matrix of the sample material is dark chocolate, which is known to be stable for years because of its low water content. The storage stability and durability of the samples (microbial spoilage) was thus ensured during the investigation period under the specified storage conditions.

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. 0,42 (17,5°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 42nd week of 2020. The testing method was optional. The tests should be finished at 11th December 2020 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** and/or **Pecan** in the range of mg/kg in the matrix of **Chocolate**. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The **"spiking level sample"** contains the allergens in a simple matrix in **similar amounts** without further processing and should be analysed like a normal sample.*

Please note the attached information on the proficiency test.
(see documentation, section 5.4 Information on the PT)

2.3 Submission of results

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

All 13 participants submitted their results in time.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD	RSD_r	RSD_R	σpt	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 24 – 42% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

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

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

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

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

ELISA results given as **hazelnut protein** or **pecan protein** were converted by DLA to **total food items (hazelnut, pecan)** using the analyzed protein content of the raw materials (see page 5).

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

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

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

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

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

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

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

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

4.1 Proficiency Test Hazelnut

4.1.1 ELISA Results: Hazelnut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
11	negative	0	positive	27,5	2/2 (100%)	BF	
1	negative	< BG	positive	12,3	2/2 (100%)	ES	Result converted °
6	positive	46	positive	135	1/2 (50%)	IL	Result converted °
7	negative	<1,1	positive	5,39	2/2 (100%)	MI	Result converted °
2	negative	<2,5	positive	19,5	2/2 (100%)	RS-F	
3	negative		positive	34,0	2/2 (100%)	RS-F	
8	negative	< 2,5	positive	37,2	2/2 (100%)	RS-F	
10	negative	<2.5	positive	32,5	2/2 (100%)	RS-F	
13	negative	<2,5	positive	13,9	2/2 (100%)	RS-F	
12	negative	<1	positive	24,0	2/2 (100%)	SP	
4	negative	0	positive	13,6	2/2 (100%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	1		11	
Number negative	10		0	
Percent positive	9		100	
Percent negative	91		0	
Consensus value	negative		positive	

Methods:

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

Comments:

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

A positive result for sample A was obtained with the method IL (Immuno-lab).

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Hazelnut	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
11	27,5	1,0		BF	
1	12,3	-1,8		ES	Result converted °
6	135	20,6		IL	Result converted °, Outlier excluded
7	5,39	-3,0		MI	Result converted °
2	19,5	-0,5	-1,2	RS-F	
3	34,0	2,2	1,0	RS-F	
8	37,2	2,8	1,4	RS-F	
10	32,5	1,9	0,7	RS-F	
13	13,9	-1,5	-2,0	RS-F	
12	24,0	0,4		SP	
4	13,6	-1,5		VT	

° calculation see p. 19

Methods:

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

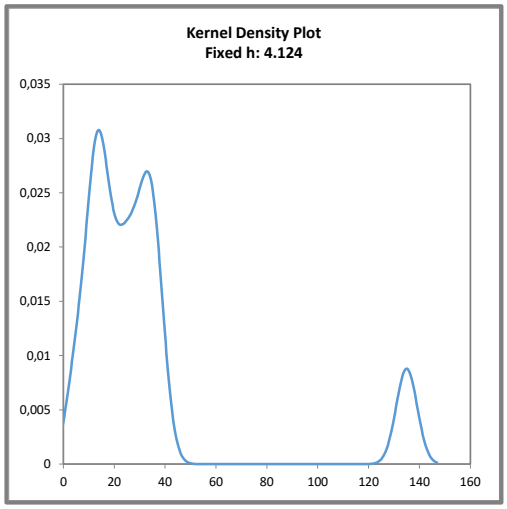


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimate showed a distribution with two peaks that could not be clearly attributed to individual methods. A side peak at about 130 mg/kg is related to an outlier outside the target range.

Characteristics: Quantitative evaluation ELISA Hazelnut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results°	10	5
Number of outliers	1	0
Mean	22,0	27,4
Median	21,7	32,5
Robust Mean (X_{pt})	**22,0**	**27,4**
Robust standard deviation (S*)	**12,1**	**11,5**
Target range:		
Target standard deviation σ_{pt}	**5,50**	**6,85**
lower limit of target range	**11,0**	**13,7**
upper limit of target range	**33,0**	**41,1**
Quotient S/σ_{pt}*	*2,2*	*1,70*
Standard uncertainty $U_{(Xpt)}$	*4,80*	*6,43*
Results in the target range	*7*	*5*
Percent in the target range	*70*	*100*

° without result No. 6 (excluded in advance)

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a two-peaked distribution without clear method-dependent differences.

The evaluation of all methods and the evaluation of results from method RS-F showed a normal to low variability of results, with quotients S/σ_{pt} slightly above or 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 68% and 85% of the spiking level of hazelnut to sample B and thus within the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for Hazelnut").

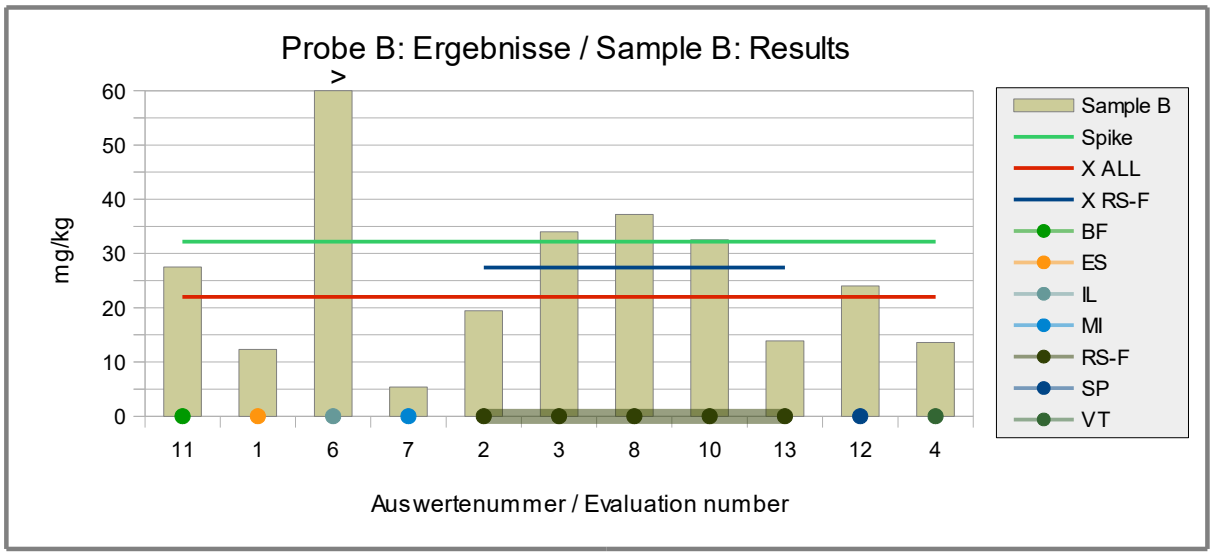


Abb./Fig. 2: ELISA Results Hazelnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

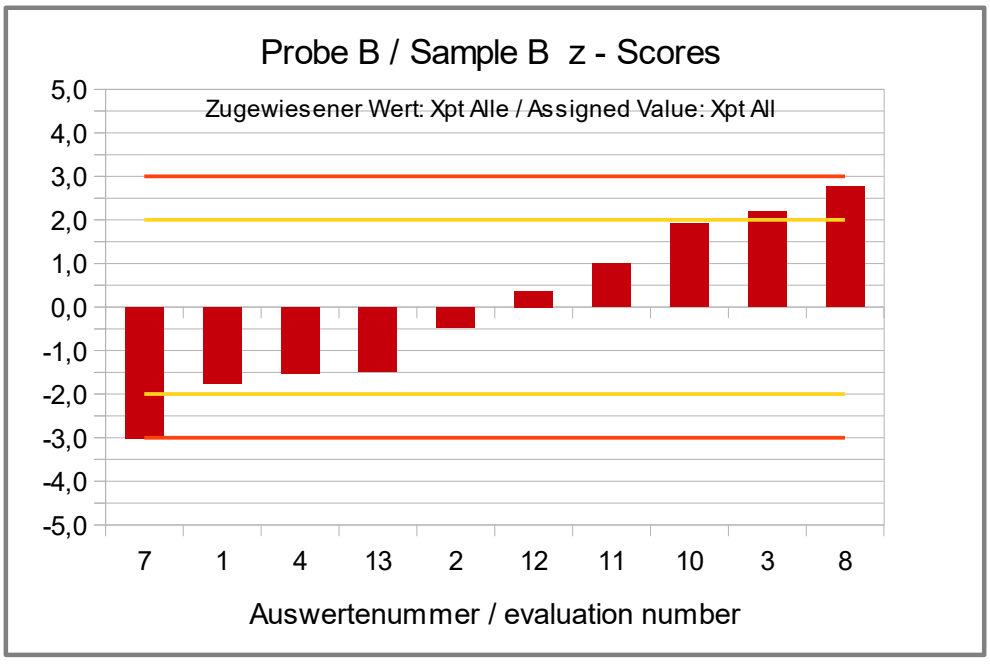


Abb./Fig. 3:
z-Scores (ELISA Results Hazelnut)
Assigned value: robust mean of all results

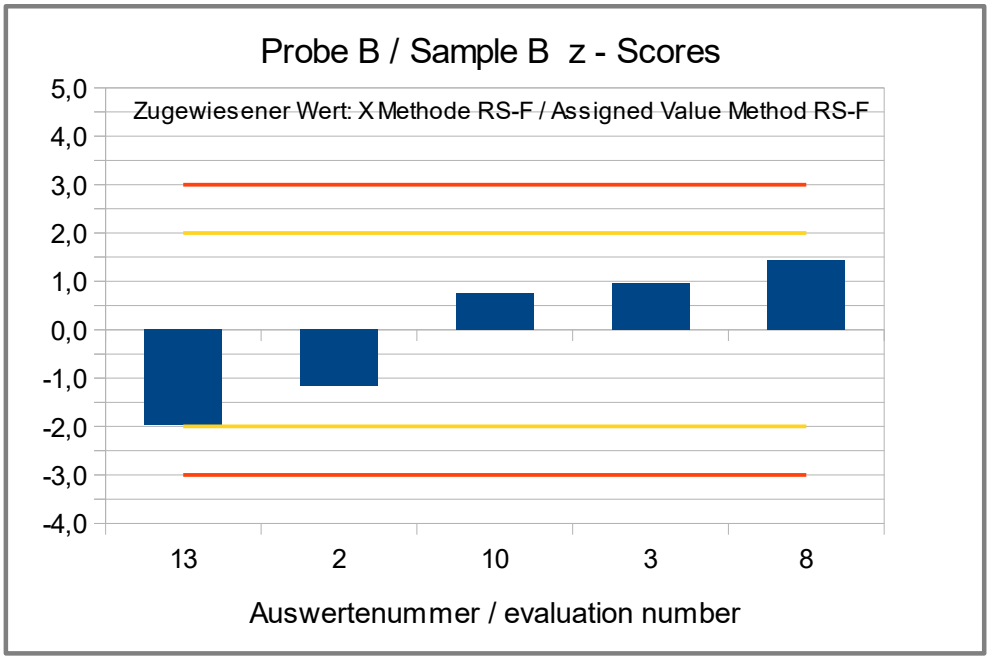


Abb./Fig. 4:
z-Scores (ELISA Results Hazelnut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Hazelnut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
11	35,6	0,22	BF	
1	12,9	-2,5	ES	Result converted °
6	201	20	IL	Result converted °, Outlier excluded
7	21,3	-1,5	MI	Result converted °
2	29,3	-0,52	RS-F	
3	60,0	3,1	RS-F	
8	45,6	1,4	RS-F	
10	43,2	1,1	RS-F	
13	>20		RS-F	
12	35,0	0,15	SP	
4	23,7	-1,2	VT	

° calculation see p. 19

Methods:

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

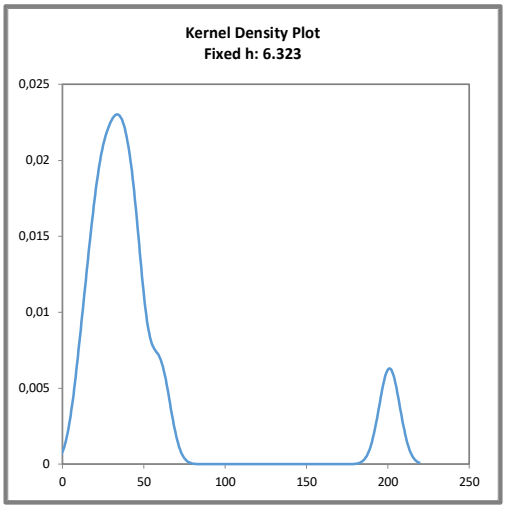


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a shoulder at 60 mg/kg and a side peak at about 200 mg/kg due to a single value outside the target range.

Characteristics: Quantitative evaluation ELISA Hazelnut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results°	9
Number of outliers	1
Mean	34,1
Median	35,0
Robust Mean (X_{pt})	**33,7**
Robust standard deviation (S*)	**15,4**
Target range:	
Target standard deviation σ_{pt}	**8,43**
lower limit of target range	**16,9**
upper limit of target range	**50,6**
Quotient S/σ_{pt}*	*1,8*
Standard uncertainty U(X_{pt})	*6,42*
Results in the target range	*7*
Percent in the target range	*78*

° without result No. 6 (excluded in advance)

Comments to the statistical characteristics and assigned values:

The kernel density estimate showed an nearly symmetrical distribution with one high single value (outlier).

The evaluation of all methods showed a normal variability of results, with a quotient S/σpt 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 mean of the evaluation was 78% of the spiking level of hazelnut to the spiking level sample and were within the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for hazelnut").

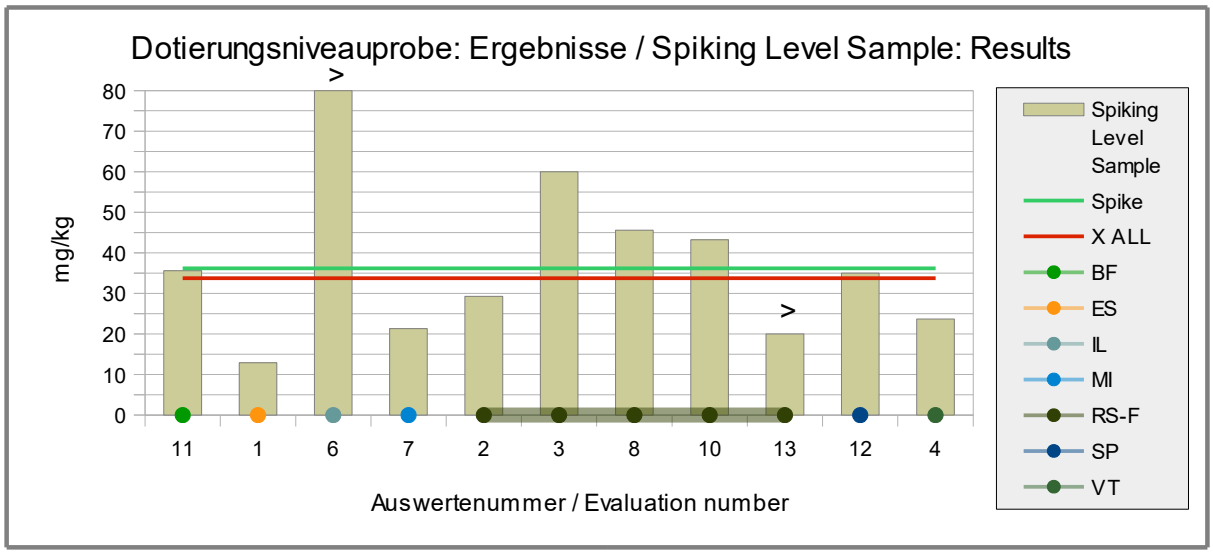


Abb./Fig. 6: ELISA Results Hazelnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

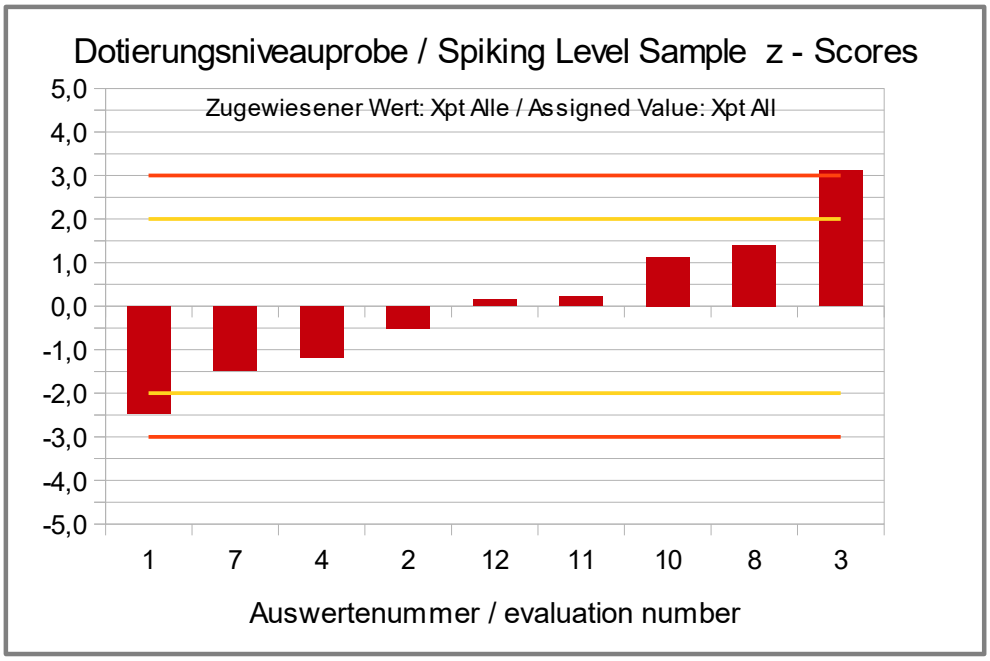


Abb./Fig. 7:
z-Scores (ELISA Results Hazelnut)
Assigned value: robust mean of all results

Recovery Rates with z-scores ELISA for Hazelnut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
11	35,6	**98**	-0,07	27,5	**85**	-0,58	BF	
1	12,9	36	-2,6	12,3	38	-2,5	ES	Result converted °
6	201	555	18	135	419	13	IL	Result converted °, Outlier excluded
7	21,3	**59**	-1,6	5,39	17	-3,3	MI	Result converted °
2	29,3	**81**	-0,76	19,5	**60**	-1,6	RS-F	
3	60,0	166	2,6	34,0	**106**	0,22	RS-F	
8	45,6	**126**	1,0	37,2	**116**	0,62	RS-F	
10	43,2	**119**	0,77	32,5	**101**	0,04	RS-F	
13	>20			13,9	43	-2,3	RS-F	
12	35,0	**97**	-0,13	24,0	**75**	-1,0	SP	
4	23,7	**65**	-1,4	13,6	42	-2,3	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Anzahl im AB	**7**	Anzahl im AB	**6**
Prozent im AB	**70**	Prozent im AB	**55**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

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

Comments:

70% (7) of the participants obtained a recovery rate in the range of the AOAC-recommendation of 50-150% with the spiking level sample by ELISA. For the spiked food matrix sample B, 55% (6) of the recovery rates were within the range of acceptance.
The associated z-scores are based on the target standard deviation of 25%.

4.1.2 PCR Results: Hazelnut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Übereinstimmungen mit Konsenswerten		
7	negative		positive		2/2 (100%)	ASU	
9	negative		negative		1/2 (50%)	CEN	
1	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA	
10	negative	< 1	positive	26,0	2/2 (100%)	SFA	

	Sample A		Sample B	
Number positive	0		4	
Number negative	5		1	
Percent positive	0		80	
Percent negative	100		20	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

A negative result for sample B was obtained using CEN (European Committee for Standardization Method).

Quantitative Valuation PCR: Sample B

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

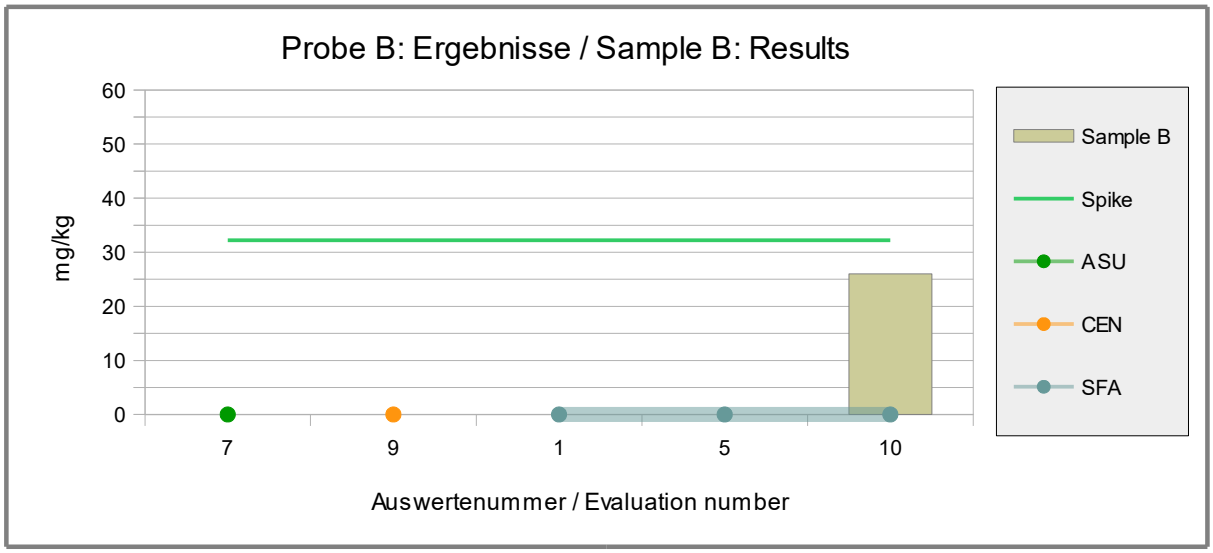


Abb./Fig. 8:
PCR Results Hazelnut
green line = Spiking level
round symbols = Applied methods (see legend)

Note:
Kernel density estimation was not carried out due to the number of < 8 results.

Quantitative Valuation PCR: Spiking Level Sample

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

Evaluation number	Hazelnut	Hazelnut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positive			ASU	
9	negative			CEN	
1	positive			SFA	
5	positive			SFA	
10	positive	77,1		SFA	

Number positive	4
Number negative	1
Percent positive	80
Percent negative	20
Consensus value	positiv

Methods:
ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comment:
80% positive results were obtained for the spiking level sample.

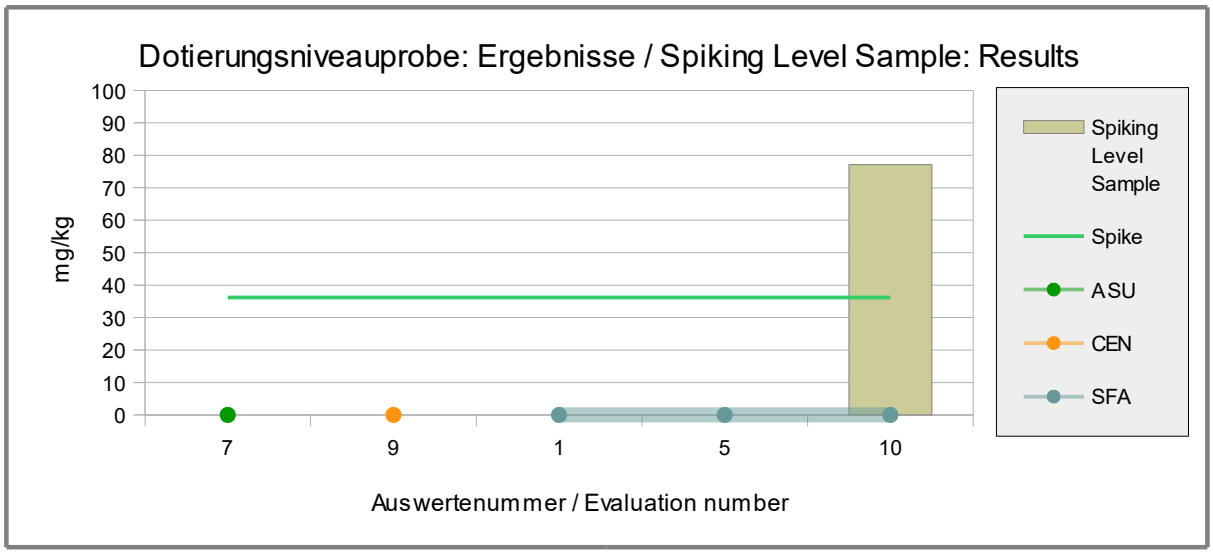


Abb./Fig. 9: PCR Results Hazelnut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Hazelnut:
Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
7							ASU	
9							CEN	
1							SFA	
5							SFA	
10	77,1	213	4,5	26,0	**81**	-0,77	SFA	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comments:
One participant submitted quantitative results by PCR and obtained recovery rates within the range of the AOAC-recommendation of 50-150% for the spiked food matrix sample B.
The associated z-scores are based on the target standard deviation of 25%.

4.2 Proficiency Test Pecan

4.2.1 ELISA Results: Pecan

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
2	negative	< 6,48	positive	9,13	2/2 (100%)	3M	Result converted °
11	negative	0	positive	39,4	2/2 (100%)	BF	
10	negative	< 2	positive	9,26	2/2 (100%)	DE	
7	negative	< 2	positive	11,0	2/2 (100%)	SP	
12	negative	< 2	positive	22,0	2/2 (100%)	SP	
13	positive	2,5	positive	12,6	1/2 (50%)	SP	

° calculation see p. 19

	Sample A		Sample B	
Number positive	1		6	
Number negative	5		0	
Percent positive	17		100	
Percent negative	83		0	
Consensus value	negative		positive	

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

DE = Demeditec ELISA

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

One positive result for sample A was obtained using method SP (SensiSpec).

Quantitative valuation of ELISA-results: Sample B

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

Evaluation number	Pecan	z-Score Xpt_{ALL}	z-Score $Xpt_{PEAK\ 11}$	Method	Remarks
	[mg/kg]				
2	9,13	-1,6	-0,7	3M	Result converted °
11	39,4	6,1		BF	
10	9,26	-1,6	-0,6	DE	
7	11,0	-1,2	0,0	SP	
12	22,0	1,7	4,0	SP	
13	12,6	-0,8	0,6	SP	

° calculation p. 19

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

DE = Demeditec ELISA

SP = SensiSpec ELISA Kit, Eurofins

Note:
Due to the low number of < 8 results a kernel density estimation could not be carried out.

Characteristics: Quantitative evaluation ELISA Pecan

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

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method Peak 11** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{PEAK\ 11}}}$
Number of results	6	5
Number of outliers	–	–
Mean	17,2	12,8
Median (X_{pt})	11,8	**11,0**
Robust Mean (X_{pt})	**15,5**	12,0
Robust standard deviation (S*)	**9,32**	**4,09**
Target range:		
Target standard deviation σ_{pt}	**3,88**	**2,75**
lower limit of target range	**7,77**	**5,50**
upper limit of target range	**23,3**	**16,5**
Quotient S/σ_{pt}*	*2,4*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*4,76*	*2,28*
Results in the target range	*5*	*4*
Percent in the target range	*83*	*80*

Methods:
Peak 11 = 3M, Demeditec, SensiSpec

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} abow 2,0.*
The distribution of the results of "peak 11" (without result no. 11 from method BF) showed a normal variability with a quotient S/σ_{pt} below 2,0. The median was used as the assigned value.*

The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited, because there were only a few results for some methods. The target values do not apply to the single methods.

The robust means or the median of the evaluations were 46% and 32% of the spiking level of pecan to sample B and thus below the range of the recommendations for the applied methods (s. 3.4.3 and p.43 "Recovery rates ELISA for pecan")

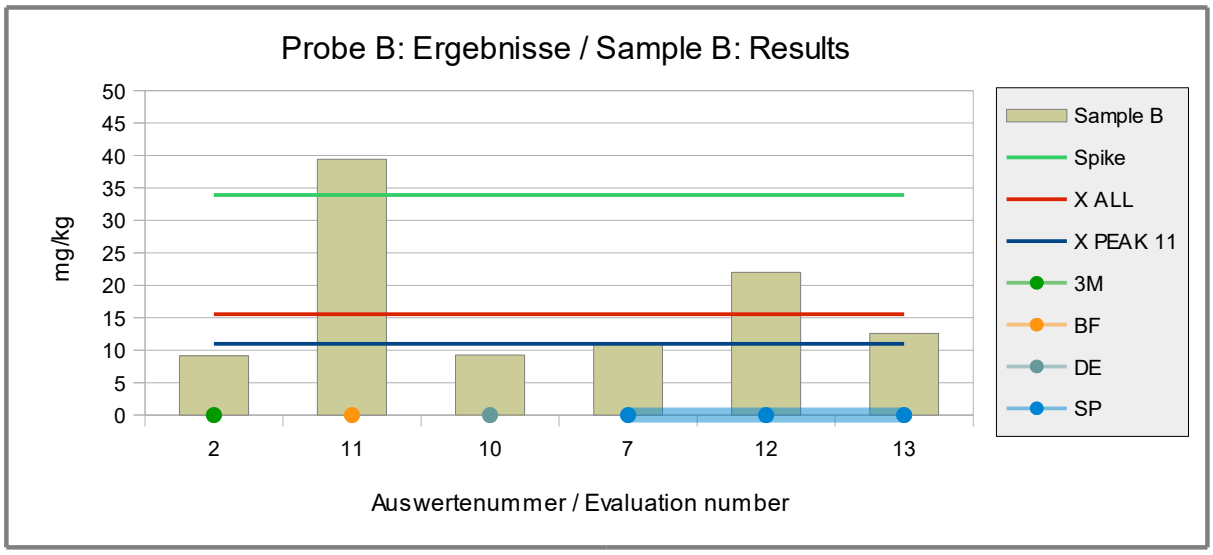


Abb./Fig. 10: ELISA Results Pecan
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value median results of "peak 11"
round symbols = Applied methods (see legend)

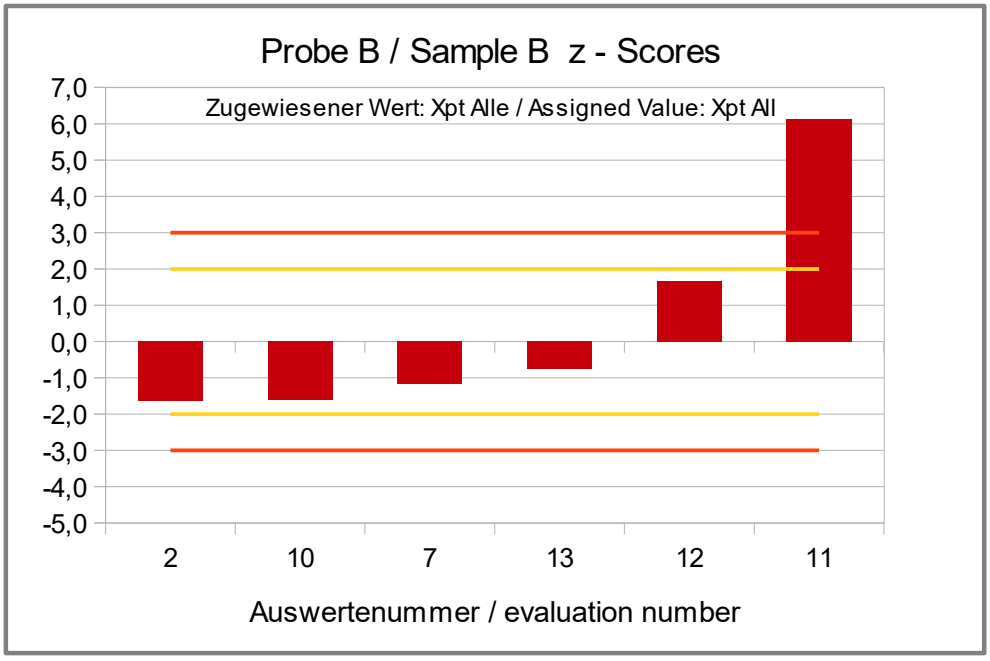


Abb./Fig. 11:
z-Scores (ELISA Results Pecan)
Assigned value robust mean of all results

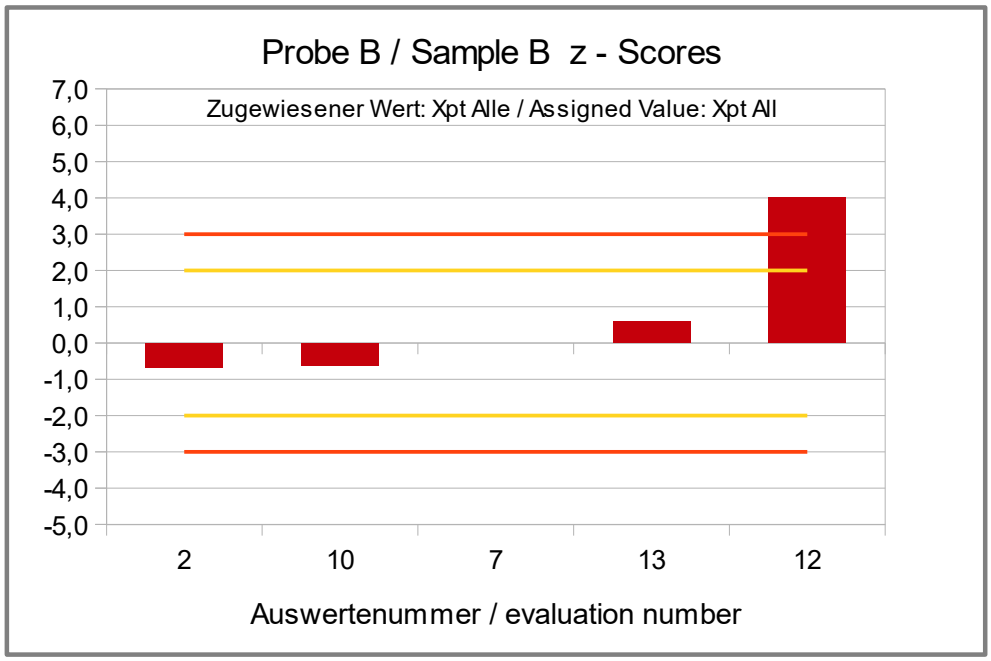


Abb./Fig. 12:
z-Scores (ELISA Results Pecan)
Assigned value median of results of "peak 11"

Quantitative valuation of ELISA: Spiking Level Sample

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

Evaluation number	Pecan	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	21,4	-2,9	3M	Result converted °
11	116	1,9	BF	
10	100	1,1	DE	
7	96,0	0,9	SP	
12	60,0	-1,0	SP	
13	>60		SP	

° Calculation p. 19

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

DE = Demeditec ELISA

SP = SensiSpec ELISA Kit, Eurofins

Note:
Due to the low number of < 8 results a kernel density estimation could not be carried out.

Characteristics: Quantitative evaluation ELISA Pecan

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

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	78,7
Median	96,0
Robust Mean (X_{pt})	**78,7**
Robust standard deviation (S*)	**43,1**
Target range:	
Target standard deviation σ_{pt}	**19,7**
lower limit of target range	**39,4**
upper limit of target range	**118**
Quotient S/σ_{pt}*	*2,2*
Standard uncertainty $U_{(X_{pt})}$	*24,1*
Results in the target range	*4*
Percent in the target range	*80*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a slightly increased variability of results, with a quotient S/σ_{pt} abow 2,0.*

The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited, because there were only a few results for some methods. The target value does not apply to the single methods.

The robust mean of the evaluation was 224% of the spiking level of pecan to the spiking level sample and thus above the range of the recommendations for the applied methods (s. 3.4.3 and p.43 "Recovery rates ELISA for pecan").

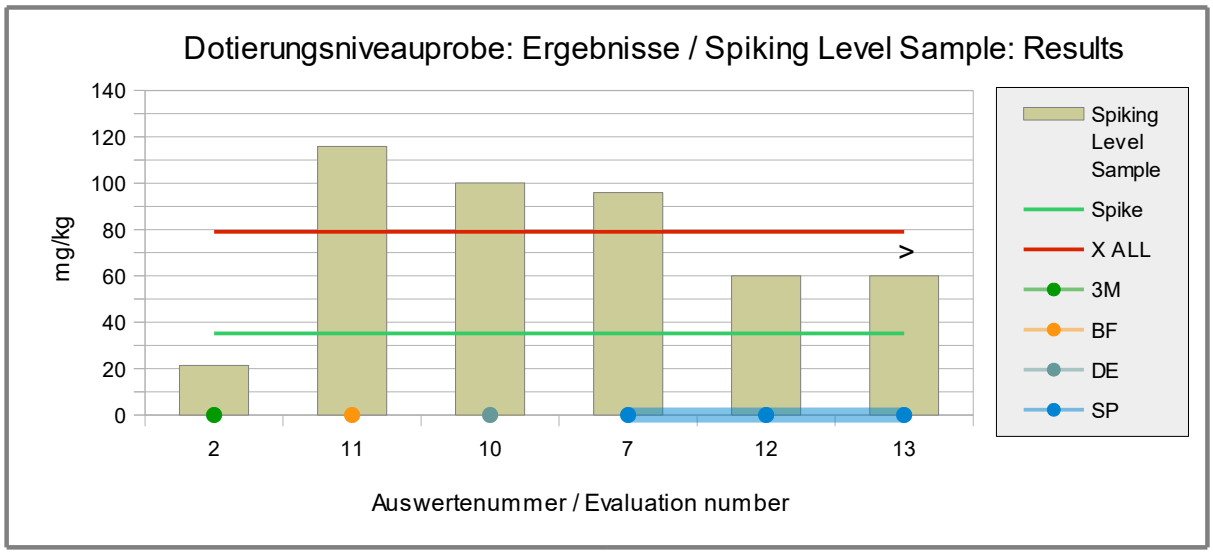


Abb./Fig. 13: ELISA Results Pecan
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

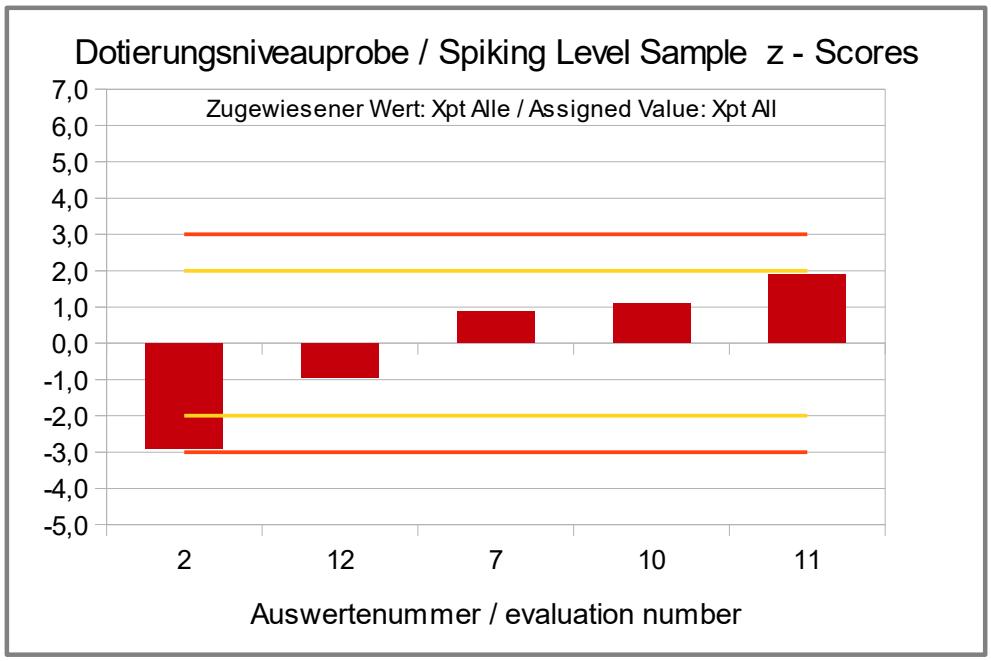


Abb./Fig. 14:
z-Scores (ELISA Results Pecan)
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA for Pecan: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
2	21,4	**61**	-1,6	9,13	27	-2,9	3M	Result converted °
11	116	329	9,2	39,4	**116**	0,65	BF	
10	100	285	7,4	9,26	27	-2,9	DE	
7	96,0	273	6,9	11,0	32	-2,7	SP	
12	60,0	170	2,8	22,0	**65**	-1,4	SP	
13	>60			12,6	37	-2,5	SP	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	2
Percent in RA	20	Percent in RA	33

Methods:
3M = 3M Protein ELISA Kit
BF = MonoTrace ELISA, BioFront Technologies
DE = Demeditec ELISA
SP = SensiSpec ELISA Kit, Eurofins

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
One participant obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B two of the recovery rates were within the range of acceptance.
The associated z-scores are based on the target standard deviation of 25%.

4.2.2 PCR Results: Pecan

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
1	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA	
10	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA-4p	
3	negative		positive		2/2 (100%)	div	
7	negative		positive		2/2 (100%)	div	

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

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative Valuation of PCR: Spiking level sample

No quantitative valuation was done, because there were none available.

Evaluation number	Pecan	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positive			SFA	
5	positive			SFA	
10	positive			SFA	
5	positive			SFA-4p	
3	positive			div	
7	positive			div	

Number positivee	6
Number negativee	0
Percent positivee	100
Percent negativee	0
Consensus value	positive

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

4.3 Participant z-Scores: overview table

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

Evaluation number	ELISA Haselnut: **Xpt** (div. Methods)		ELISA Haselnut: **Xpt** (Method: RS-F)		ELISA Pecan: **Xpt** (div. Methods)		ELISA Pecan: **Xpt** (Methods: Peak 11)	
	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1	-1,8	-2,5						
2	-0,46	-0,52	-1,2		-1,6	-2,9	-0,68	
3	2,2	3,1	1,0					
4	-1,5	-1,2						
5								
6	21	20						
7	-3,0	-1,5			-1,2	0,88	0,00	
8	2,8	1,4	1,4					
9								
10	1,9	1,1	0,75		-1,6	1,1	-0,63	
11	1,0	0,22			6,1	1,9		
12	0,37	0,15			1,7	-1,0	4,0	
13	-1,5		-2,0		-0,75		0,58	

Methods: RS-F = Ridascreen® Fast, R-Biopharm
Peak 11 = 3M, Demeditec, SensiSpec

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 Haselnut: **Xpt** (div. Methods)		ELISA Pecan: **Xpt** (div. Methods)		PCR Haselnut: **Xpt** (div. Methods)	
	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1	-2,5	-2,6				
2	-1,6	-0,76	-2,9	-1,6		
3	0,22	2,6				
4	-2,3	-1,4				
5						
6	13	18				
7	-3,3	-1,7	-2,7	6,9		
8	0,62	1,0				
9						
10	0,04	0,77	-2,9	7,4	-0,77	4,5
11	-0,58	-0,07	0,65	9,2		
12	-1,0	-0,13	-1,4	2,8		
13	-2,3		-2,5			

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. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
BF	11	11.12.20	negative	0	positive	27,5	positive	35,6	0,04	1		Hazelnut	MonoTrace Hazelnut ELISA kit, BioFront Technologies
ES	1	21.10.20	-	< BG	-	1,73	-	1,81		0,5		Hazelnutprotein	ELISA Systems Hazelnut ESHRD-48
IL	6	28.10.2020r	positive	6,5	positive	19	positive	28,25	0,3	1	8,93	Hazelnutprotein	Immunolab Hazelnut ELISA
MI	7	5.11.	negative	< 0,16	positive	0,76	positive	3	0,16	0,16		Hazelnutprotein	Morinaga Hazelnut ELISA Kit II
RS-F	2	12.11.20	negative	<2,5	positive	19,45	positive	29,3		2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	3	27.10.	negative		positive	34	positive	60		2,5	20	Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	8	26.10.20	-	< 2,5	-	37,2	-	45,6	0,19	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	10	17.11.20	negative	< 2.5	positive	32,54	positive	43,21	2,5	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	13	20.01.21	negative	< 2,5	positive	13,9	positive	> 20	0,19	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
SP	12	20.11.20	negative	< 1	positive	24	positive	35	0.3	1		Hazelnut	Eurofins SensiSpec Hazelnut ELISA Kit
VT	4	03.12.20	-	0	-	13,6	-	23,7				Hazelnut	Veratox Hazelnut, Neogen

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Hazelnut:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BF	11	Monoclonal antibody	1:20 extraction ratio/10 min/60°C	no	5% non-fat dry milk added to 1X extraction buffer
ES	1	Anti-Hazelnut	As Per Kit Instructions	yes	
IL	6	Hazelnut protein	Extraction: 1 g of homogenized mixture suspended in 20 mL of pre-diluted extraction and sample dilution buffer/ 15 minutes of sample incubation in 60°C/10 minutes of 2000 x g centrifugation Determination: 100 µL of particle-free solution, ready-to-use standards applied per well/ 20 minutes incubation at room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL conjugate into each well/ 20 minutes incubation in room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL substrate solution into each well/ 20 minutes incubation in the dark, at room temperature/ add 100 µL Stop enzyme solution into each well/ Measure absorbance at 450 nm (reference 620 nm)	No	-
MI	7	recognizes hazelnut proteins	As Per Kit Instructions	yes	
RS-F	2		as stipulated in kit insert	yes	recovery in sample A: 124 %
RS-F	3		Allergen extraction buffer(kit) with skimmed milk powder	yes	
RS-F	8			yes	
RS-F	10	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	13		with skimmed milk powder		
SP	12		addition of confidential additive		
VT	4		15 min / 60°C	no	

5.1.2 ELISA: Pecan

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
3M	2	12.11.20	negative	< 0,667	positive	0,94	positive	2,2		0,67		Pecan Protein	3M Pecan Protein ELISA Kit E96PEC
BF	11	11.12.20	negative	0	positive	39,4	positive	115,9	0,17	1		Pecan	MonoTrace Pecan ELISA kit, BioFront Technologies
DE	10	20.11.20	negative	< 2	positive	9,26	positive	100,15	2	2		Pecan	
SP	7	27.10.20	negative	< 2	positive	11	positive	96	2	2		Pecan	Eurofins SensiSpec Pecan ELISA Kit
SP	12	20.11.20	negative	< 2	positive	22	positive	60	0.2	2		Pecan	Eurofins SensiSpec Pecan ELISA Kit
SP	13	20.01.21	positive	2,5	positive	12,6	positive	> 60	0,2	2		Pecan	Eurofins SensiSpec Pecan ELISA Kit

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
3M	2		as stipulated in kit insert	yes	recovery in sample A: 52 % recovery in sample B: 49 %
BF	11	Monoclonal antibody	1:10 extraction ratio/10 min/60° C	no	5% non-fat dry milk added to 1X extraction buffer
DE	10	As Per Kit Instructions	As Per Kit Instructions	Yes	Demeditec Kit
SP	7	recognizes pecan proteins	As Per Kit Instructions	yes	
SP	12		addition of confidential additive		
SP	13				

5.1.3 PCR: Hazelnut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
ASU	7	10.11.	negative		positive		positive		10			Hazelnut-DNA	ASU §64 Methode/method
CEN	9		negative		negative		negative		< 10			Allergen DNA	SRPS CEN/TS 15634-3
SFA	1	03.11.20	negative		positive		positive					Hazelnut-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	21.10.20	negative		positive		positive		0,4		30	Hazelnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10	28.10.20	negative	< 1	positive	26	positive	77,08	1	1	40	Hazelnut	Sure Food ALLERGEN, R-Biopharm / Congen

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ASU	7		CTAB / Priteinase K / Rnase A / Maxwell / Real-time PCR 45 Cycles	yes	§64 LFGB L 44.00-08:2010
CEN	9	according to SRPS CEN/TS 15634-3	1. DNA Extraction with CTAB Aria Mx Real time PCR System, Agilent Technologies, 45 cycles		
SFA	1	characteristic sequence section of the hazelnut DNA	Dotierungsprobe: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Zyklen Probe A+B: Dneasy Mericon Food-Kit,QIAquick PCR Purification-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 Zyklen	yes	
SFA	5	Corylus	Extraktion mittels SureFood® Prep Advanced Protokoll 1 (S1053)	yes	K01
SFA	10	As Per Kit Instructions	As Per Kit Instructions	No	

5.1.4 PCR: Pecan

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
SFA	1	04.11.20	negative		positive		positive					Pecan-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	21.10.20	negative		positive		positive		0,4		30	Pecan	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10	28.10.20	negative		positive		positive		1			Pecan	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	5	21.10.20	negative		positive		positive		0,4		30	Pecan	Sure Food Allergen 4plex, R-Biopharm / Congen
div	3	29.10.	negative		positive		positive		5			Pecan-DNA	Literaturmethode
div	7	10.11.	negative		positive		positive		1			Pecan-DNA	interne Methode

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
SFA	1	characteristic sequence section of pecan DNA	Spiking level sample: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles Sample A+B: Dneasy Mericon Food-Kit,QIAquick PCR Purification-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
SFA	5	Carya illinoinensis	Extraction using SureFood® Prep Advanced Protokoll 1 (S1053)	yes	K01, QE to Scaly bark hickory (Carya ovata) 100 %.
SFA	10	As Per Kit Instructions	As Per Kit Instructions	No	
SFA-4p	5	Carya illinoinensis	Extraction using SureFood® Prep Advanced Protokoll 1 (S1053)	yes	K02, QE to Scaly bark hickory (Carya ovata) 100 %.
div	3		CTAB lysis using Prot. K, 60°C (O/N); phenol/chloroform extraction; clean up: FFS Kit (Promega), or Mericon Food Kit (Qiagen).		
div	7		CTAB / Priteinase K / Rnase A / Maxwell / Real-time PCR 45 Cycles	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA-ptAL06 Spiking Level Sample

Weight whole sample	1,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	50	19,9
2	5,01	57	22,8
3	4,97	64	25,8
4	5,05	52	20,6
5	5,00	60	24,0
6	4,97	50	20,1
7	5,04	57	22,6
8	4,96	53	21,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	55,4	Particles
Standard deviation	5,10	Particles
χ^2 (CHI-Quadrat)	3,29	
Probability	**86**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	22,1	mg/kg
Standard deviation	2,04	mg/kg
rel. Standard deviaton	9,2	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,9**	
Recovery rate	112	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptAL06 - 2020***
PT name	***Allergens VI: Hazelnut and Pecannut in Chocolate with "Spiking Level Sample“***
Sample matrix (processing)	***Samples A + B:*** *Chocolate 70%/ ingredients: Cocoa mass, sugar, cocoa butter, defatted cocoa, emulsifier: lecithins, vanilla extract other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A, B + Spiking Level Sample:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Hazelnut (Hazelnut protein, DNA), Pecan (Pecan 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 December 11th 2020***
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
		USA
		CANADA
		SWITZERLAND
		Germany
		Germany
		Germany
		POLAND
		Germany
		Germany
		SERBIA
		GREAT BRITAIN
		Germany

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

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

7. Index of references

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

Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 18.00-20 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of almond (Prunus dulcis) in rice and wheat cookies and sauce powders by PCR]
33. ASU §64 LFGB L 18.00-21 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Paranuss (Bertholletia exceisa) in Reis- und Weizenkeksen sowe in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of brazil nut (Bertholletia exceisa) in rice and wheat cookies and sauce powders by PCR]
34. ASU §64 LFGB L 18.00-22 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]