Evaluation Report

proficiency test

DLA ptAL07 (2021)

Allergens VII:

Pistachio, Mollusks and Celery

Instant Noodle Soup

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1st Correction 11/03/2022:
In the table of p. 21 the qualitative agreement of the ELISA results for participant no. 19 was given falsely. This has been corrected.

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

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EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 4 April 2022
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.
Akkreditierung *Accreditation* ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-EP-21534-01-00	nach / according to: ISO/IEC 17.043-2010 Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen Conformity Assessment - General Requirements for Proficiency Testing Die Akkreditierung gilt für den in der Urkundenanlage genannten Umfang. The accreditation is valid for the scope of the annex to the certificate of accreditation

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 a commercially available instant soup powder (broccoli cream soup) with the addition of noodles. The basic composition was the same for both samples A and B (see Table 1).
After crushing and sieving of the raw materials, the basic mixture was homogenized.

Afterwards, the **spiked sample A** was produced as follows:
The spiking materials containing the allergenic ingredients pistachio, mollusks and celery were added to an aliquot of the base matrix and the mixture was homogenized. Subsequently, basic matrix was again added in portions in further steps and in each case homogenized until the total amount was reached.
For the **spiking level sample**, the allergenic compounds mentioned above were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Broccoli Noodle Soup (powder) Ingredients: Gluten-free noodles (40%), maltodextrin, palm fat, modified starch, glucose syrup, iodized table salt, rice flour, yeast extract, 1.4% broccoli, leek, onions, stabilizer diphosphate, spinach, thickener xanthan gum, garlic, spices, vegetable juice concentrates (carrot, two belly), flavor, tomato extract, sunflower oil, lovage	99,5 g/100 g	100 g/100 g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,6 g/100 g
Pistachio untreated, ground - as pistachio* - *thereof 21,7% total protein***	 34,7 mg/kg 7,54 mg/kg	-	 32,0 mg/kg 6,96 mg/kg
Mollusks Yesso scallop, (Mizuhopecten yessoensis) freeze dried - as mollusks* - *thereof 75,7% total protein***	 68,9 mg/kg 52,2 mg/kg	-	 72,1 mg/kg 54,6 mg/kg
Celery (celery leaves) dried and ground - as celery* - *thereof 14,2% total protein***	 69,6 mg/kg 9,88 mg/kg		 71,7 mg/kg 10,2 mg/kg
Further Ingredients: *Maltodextrin, sodium sulfate and silicon dioxide*	<0,5 g/100 g	-	<0,5 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 pistachio protein and F=6,25 for mussel protein and celery protein)

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 49% and 41%, 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 1,3 each were obtained in this PT. 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 molluscs and pistachio (Immunolab and AgraQuant) (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

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

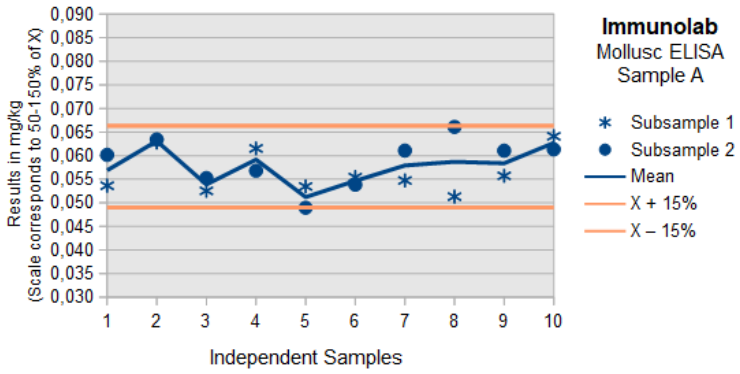
ELISA-Tests: Homogenität Mollusken / Homogeneity Molluscs

Immunolab Molluscs ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomyosin 0,0577 ± 0,0030 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	0,0536	0,0602	0,0569
2	0,0627	0,0634	0,0631
3	0,0525	0,0552	0,0539
4	0,0615	0,0568	0,0592
5	0,0535	0,0490	0,0512
6	0,0554	0,0538	0,0546
7	0,0547	0,0611	0,0579
8	0,0513	0,0661	0,0587
9	0,0557	0,0610	0,0584
10	0,0641	0,0613	0,0627

General average X	0,0577	
SD of sample means Sx	0,0037	6,4%
SD w ithin-samples Sw	0,0031	5,4%
SD betw een-samples Ss	0,0030	5,2%

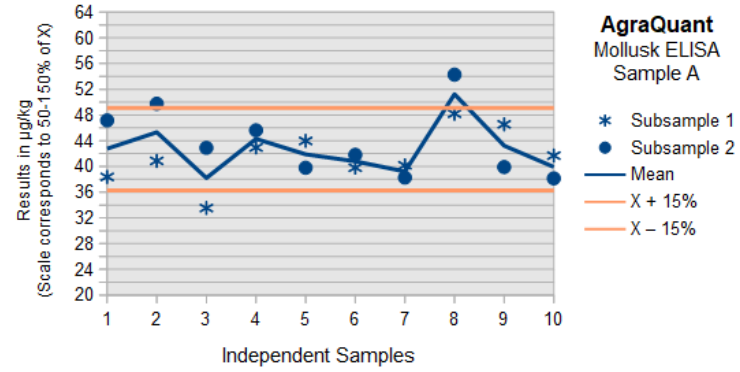


AgraQuant Mollusk ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomyosin 42,7 ± 3,1 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	38,4	47,2	42,8
2	40,9	49,8	45,3
3	33,5	42,9	38,2
4	42,9	45,6	44,3
5	44,0	39,8	41,9
6	39,8	41,8	40,8
7	40,2	38,3	39,2
8	48,2	54,3	51,2
9	46,5	39,9	43,2
10	41,7	38,2	39,9

General average X	42,7	
SD of sample means Sx	3,75	8,8%
SD w ithin-samples Sw	3,04	7,1%
SD betw een-samples Ss	3,08	7,2%



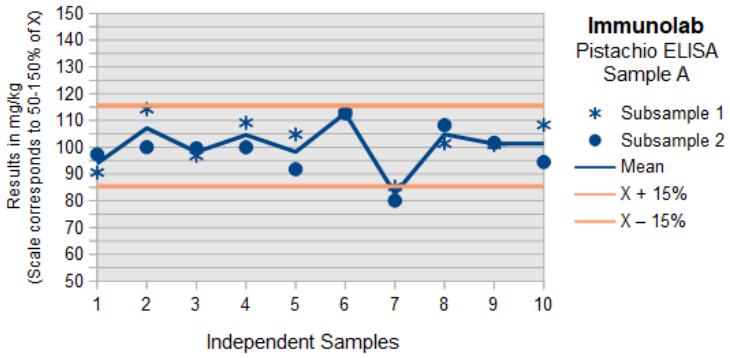
ELISA-Tests: Homogenität Pistazie / Homogeneity Pistachio

Immunolab Pistachio ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Pistachio 101 ± 7,6 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	90,6	97,4	94,0
2	114	100	107
3	96,8	100	98,2
4	109	100	105
5	105	91,8	98,3
6	113	113	113
7	85,3	80,1	82,7
8	101	108	105
9	101	102	101
10	108	94,5	101

General average X	101	
SD of sample means Sx	8,17	8,1%
SD within-samples Sw	4,41	4,4%
SD between-samples Ss	7,56	7,5%

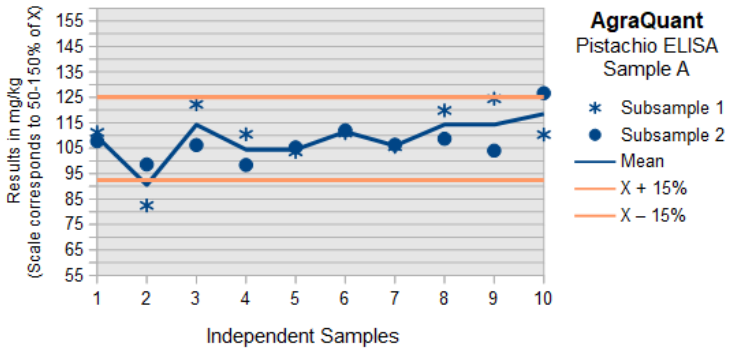


AgraQuant Pistachio ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Pistachio 109 ± 6,7 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	111	108	109
2	82,5	98,6	90,5
3	122	106	114
4	110	98	104
5	104	105	104
6	111	112	111
7	106	106	106
8	120	109	114
9	124	104	114
10	110	127	118

General average X	109	
SD of sample means Sx	7,96	7,3%
SD within-samples Sw	6,10	5,6%
SD between-samples Ss	6,69	6,1%



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 PT samples was approx. 0,40 – 0,49 (19-20°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 44th week of 2021. The testing method was optional. The tests should be finished at 07 January 2022 the latest (extended).

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 Pistachio, Mollusks and Celery in the range of mg/kg in the matrix of Instant Noodle Soup. 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 remarks 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 19 participants submitted at least one result.

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	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 18 – 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} [32-36]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
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
Celery seeds	Boiled sausage (100°C, 60min)	98,1 45,5	98,1 % 114 %	- -	12,6% 27,9%	20,7% 34,7%	18,7% 28,5%	rt-PCR ASU 08.00-65
Celery seeds	Sausage, autoclaved	10,5	10,5 %	-	25,8%	39,4%	34,9%	rt-PCR ASU 08.00-65
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soya flour	Boiled sausage (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled sausage (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59

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. One participant submitted results using the Next Generation Sequencing (NGS) method. The evaluation was carried out separately.
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 are reported then.

In the result chapter all quantitative results of the participants are displayed formatted to 3 decimal places (valid digits). 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.

The ELISA results, which were given as **pistachio protein**, were converted to the **total food item (pistachio)** using the experimentally determined protein content of the raw materials (see page 5).
The ELISA results for mollusks, which were given as **tropomyosin**, have been converted to the **protein content of the food (yesso scallop)**. For this, the tropomyosin levels were first converted to the wet weight of scallop, raw (with the factor according to the manual of the test kit manufacturers AgraQuant, SensiSpec) and by considering a water content of 80% it was converted into dry weight/yesso scallop powder. This was followed by conversion to the total protein of the mollusks/scallops using the experimentally determined protein content of the raw materials (see page 5).
In addition, a separate evaluation was carried out without conversion for the results given as tropomyosin (only quantitative).

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

4.1.1 ELISA Results: Pistachio

Qualitative valuation 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		
2	positive	21,0	negative	<4,61	2/2 (100%)	3M	result converted °
6	positive	75,6	negative	<LOQ	2/2 (100%)	AQ	
12	positive	113	negative	<LOD	2/2 (100%)	AQ	
16	negative	<1	negative	<1	1/2 (50%)	AQ	no positive sample identified
10	positive	359	negative	<4,61	2/2 (100%)	AQ-P	result converted °
13	positive	77,4	negative	<1	2/2 (100%)	BC	
5	positive	>184	negative	<4,61	2/2 (100%)	BF	result converted °
19	positive	54,0	negative	ND	2/2 (100%)	BF	
14	positive		negative		2/2 (100%)	IL	
18	positive	87,0	negative	<LOQ	2/2 (100%)	IL	
17	positive	95,0	negative	<LOQ	2/2 (100%)	SP	

° calculation see p. 19

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

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
AQ-P = AgraQuant Plus, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

Comment:
The consensus values are in qualitative agreement with the spiking of sample A. One negative result was obtained for sample A.

Quantitative evaluation of ELISA-results: Sample A

Evaluation number	Pistachio	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	21,0	-2,9	3M	result converted °
6	75,6	-0,02	AQ	
12	113	2,0	AQ	
16	<1		AQ	
10	359		AQ-P	Outlier / result converted °
13	77,4	0,08	BC	
5	>184		BF	result converted °
19	54,0	-1,2	BF	
14			IL	
18	87,0	0,58	IL	
17	95,0	1,0	SP	

° calculation see p. 19

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
AQ-P = AgraQuant Plus, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

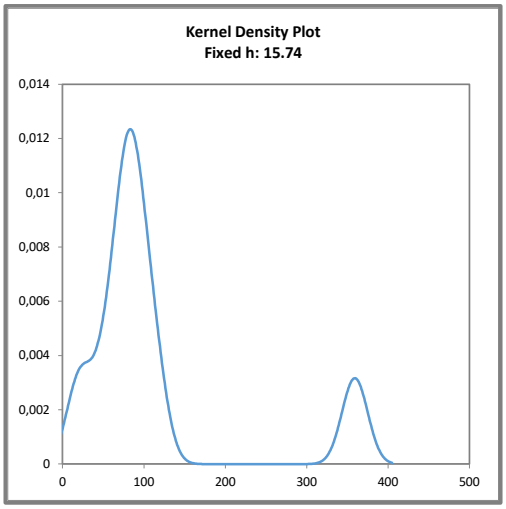


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows an approximately symmetrical distribution of the results with a shoulder at approximately < 30 mg/kg (low single value, method 3M) and a secondary peak at 359 mg/kg due to an outlier (method AQ-P).

Characteristics: Quantitative evaluation ELISA Pistachio

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	7°
Number of outliers	1
Mean	74,7
Median	77,4
Robust Mean (X_{pt})	**75,9**
Robust standard deviation (S*)	**31,1**
Target range:	
Target standard deviation σ_{pt}	**19,0**
lower limit of target range	**37,9**
upper limit of target range	**114**
Quotient S/σ_{pt}*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*14,7*
Results in the target range	*6*
Percent in the target range	*86*

° *without outlier no. 10 (excluded in advance)*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences. An outlier was excluded in advance.

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 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 219% of the spiking level of pistachio to sample A and thus above the range of the recommendations for the applied methods (see 3.4.3 and p.28 "Recovery rates with z-scores ELISA for Pistachio").

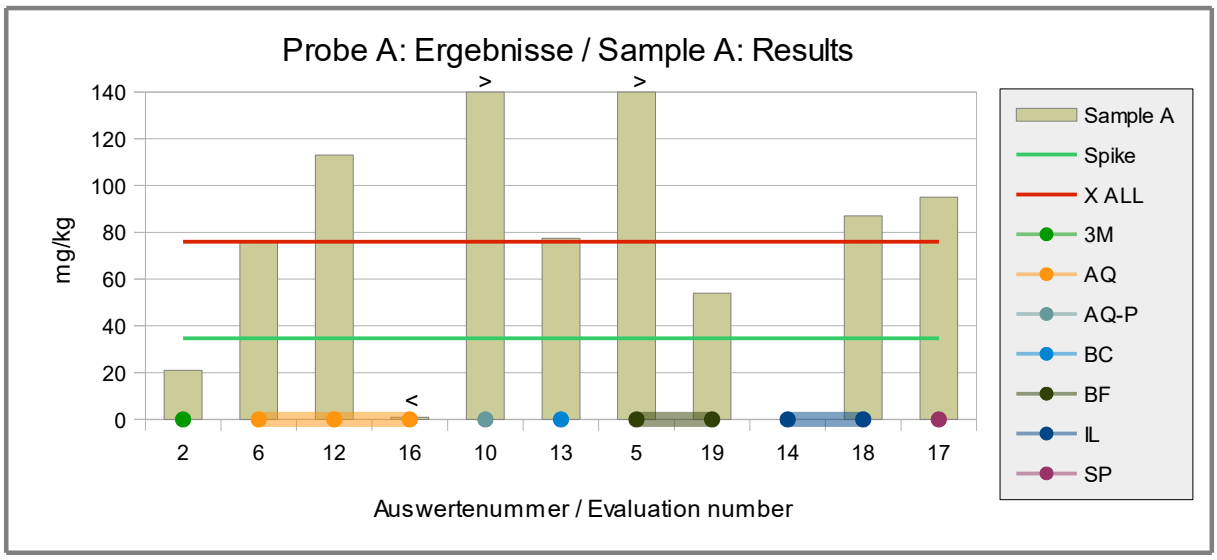


Abb./Fig. 2: ELISA Results Pistachio
green line = Spiking level (Spike)
red line = robust mean of all methods
round symbols = Applied methods (see legend)

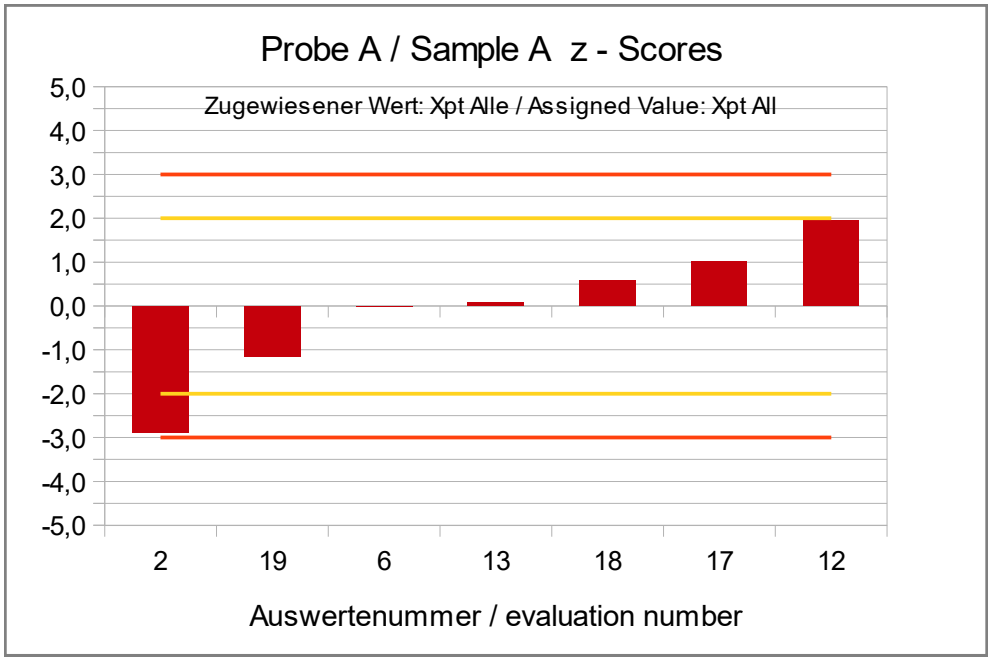


Abb./Fig. 3:
z-Scores (ELISA Results Pistachio)
Assigned value: robust mean of all results

Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Pistachio	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	16,1	-3,1	3M	result converted °
6	65,8	-0,18	AQ	
12	143	4,3	AQ	
16	<1		AQ	
10	314		AQ-P	Outlier / result converted °
13	67,3	-0,10	BC	
5	>184		BF	result converted °
19	56,0	-0,75	BF	
14			IL	
18	63,5	-0,32	IL	
17	89,0	1,2	SP	

° calculation see p. 19

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
AQ-P = AgraQuant Plus, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

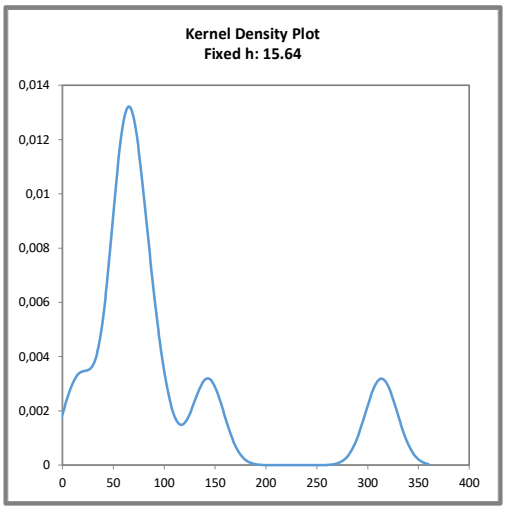


Abb. / Fig. 4:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows an approximately symmetrical distribution with 3 secondary peaks at approx. 16 mg/kg (method 3M), at 143 mg/kg (method AQ) and at 314 mg/kg (method AQ-P), which are based on 2 single values outside the target range and one outlier.

Characteristics: Quantitative evaluation ELISA Pistachio

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	7°
Number of outliers	1
Mean	71,5
Median	65,8
Robust Mean (X_{pt})	**69,0**
Robust standard deviation (S*)	**37,5**
Target range:	
Target standard deviation σ_{pt}	**17,2**
lower limit of target range	**34,5**
upper limit of target range	**103**
Quotient S/σ_{pt}*	*2,2*
Standard uncertainty $U_{(Xpt)}$	*17,7*
Results in the target range	*5*
Percent in the target range	*71*

° *without outlier no. 10 (excluded in advance)*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences. An outlier was excluded in advance.

The distribution of the results of all methods showed a slightly increased variability. The quotient S/σ_{pt} was above 2,0. The robust standard deviation is in the upper 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 mean of the evaluation was 216% of the spiking level of pistachio to the spiking level sample and was thus above the relevant requirements for the methods used (see 3.4.3 and p.28 "Recovery rates with z-scores ELISA for Pistachio").

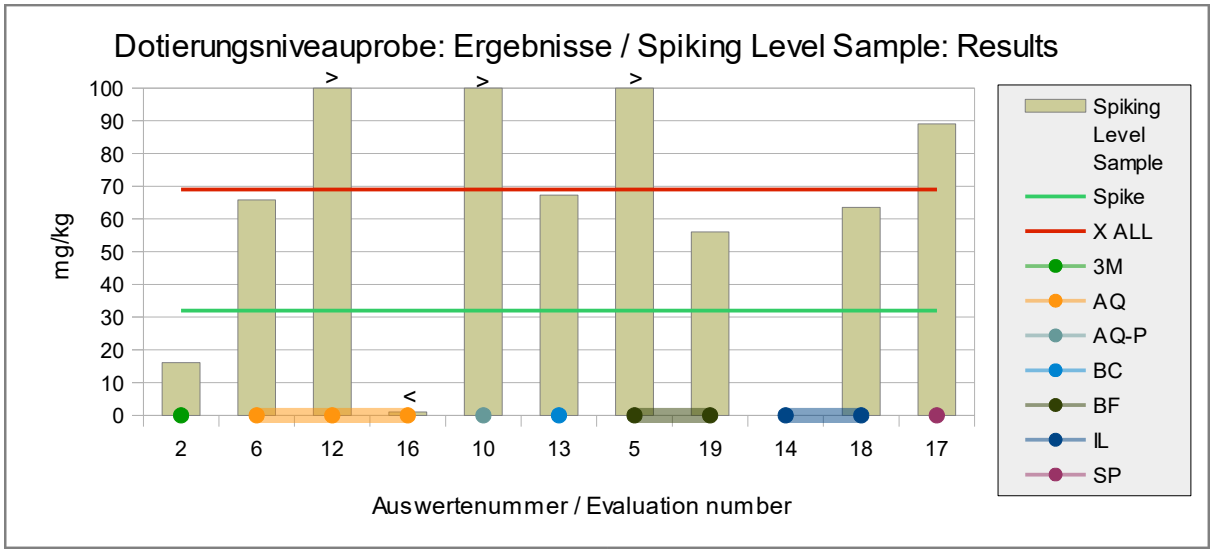


Abb./Fig. 5: ELISA Results Pistachio
green line = Spiking level (Spike)
red line = robust mean of all results
round symbols = Applied methods (see legend)

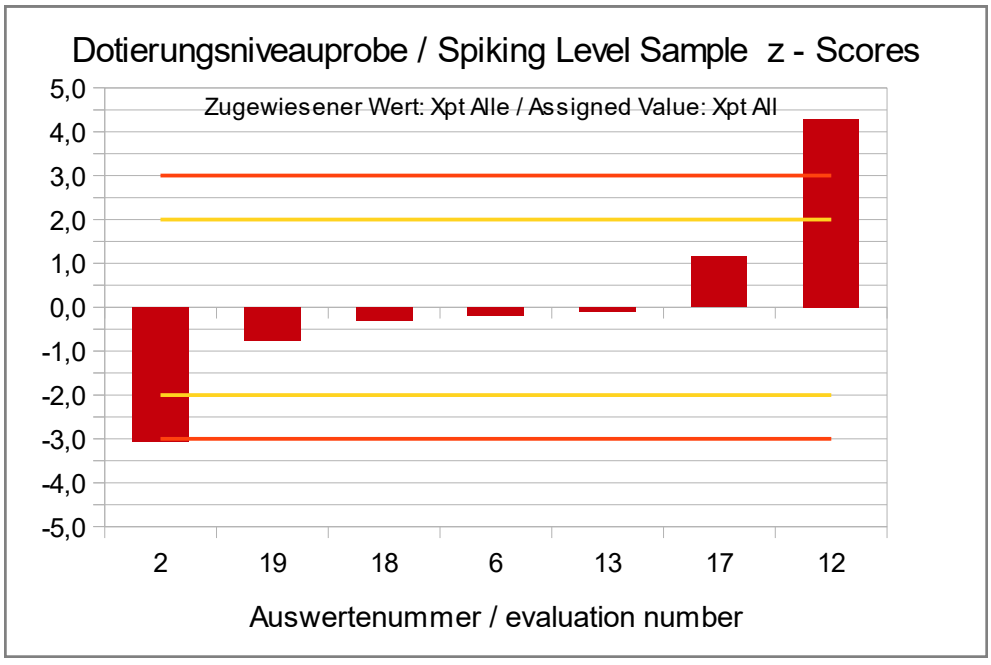


Abb./Fig. 6:
z-Scores (ELISA Results Pistachio)
Assigned value: robust mean of all results

Recovery Rates with z-Scores ELISA for Pistachio: 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}]		
2	16,1	**50,4**	-2,0	21,0	**60,5**	-1,6	3M	result converted °
6	65,8	206	4,2	75,6	218	4,7	AQ	
12	143	447	14	113	326	9,0	AQ	
16	<1			<1			AQ	
10	314	980	35	359	1035	37	AQ-P	result converted °
13	67,3	210	4,4	77,4	223	4,9	BC	
5	>184			>184			BF	result converted °
19	56,0	175	3,0	54,0	156	2,2	BF	
14							IL	
18	63,5	198	3,9	87,0	251	6,0	IL	
17	89,0	278	7,1	95,0	274	7,0	SP	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	13	Percent in RA	13

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
AQ-P = AgraQuant Plus, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
SP = SensiSpec ELISA Kit, Eurofins

Comments:

One participant (13%) obtained a recovery rate in the range of the AOAC requirement of 50-150% with the spiking level sample as well as with the spiked food matrix sample A by ELISA.
The related z-scores are based on the target standard deviation of 25%.

4.1.2 PCR Results: Pistachio

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		
8	positive		negative		2/2 (100%)	BS	
7	positive		negative		2/2 (100%)	GR	
3	positive		negative		2/2 (100%)	SFA	
10	positive		negative		2/2 (100%)	SFA	
11	positive		negative		2/2 (100%)	SFA	
13	positive	74,3	negative	<1	2/2 (100%)	SFA	
5	positive		negative		2/2 (100%)	SFA-ID	
1	positive		negative		2/2 (100%)	div	
14	positive		negative		2/2 (100%)	div	

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

Methods:
BS = qualyfast allergen, BIOSIDE
GR = SPECIALfinder Assay, real time PCR, Generon
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative evaluation PCR: Sample A

The quantitative results were not evaluated because too few single results were available.

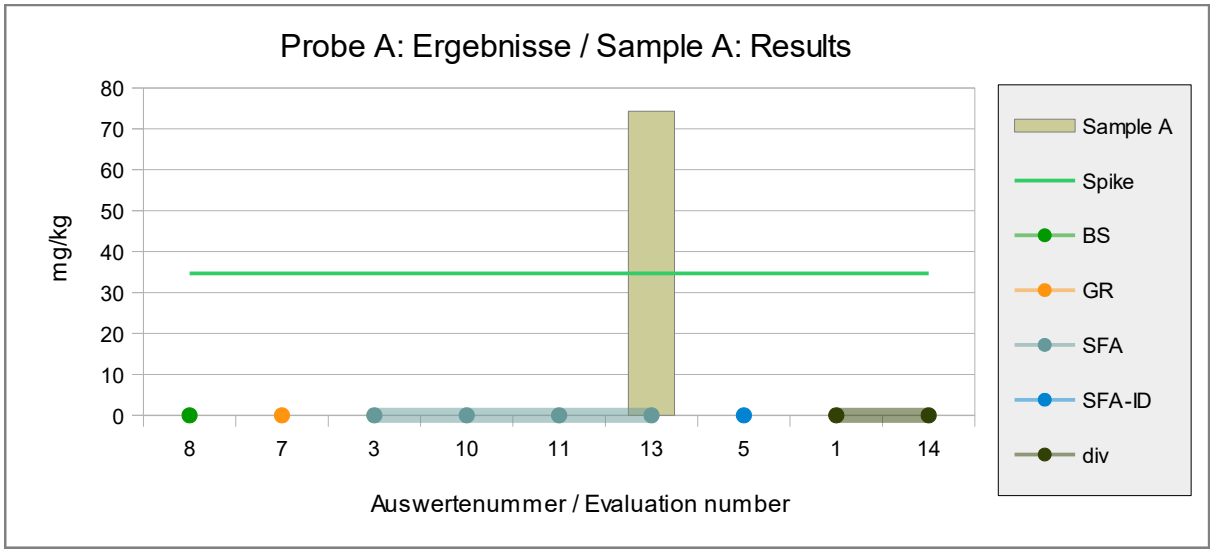


Abb./Fig. 7: PCR Results Pistachio
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative evaluation PCR: Spiking Level Sample

The quantitative results were not evaluated because too few single results were available.

Evaluation number	Pistachio	Pistachio	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
8	positive			BS	
7	-			GR	
3	positive			SFA	
10	positive			SFA	
11	positive			SFA	
13	positive	98,0		SFA	
5	positive			SFA-ID	
1	positive			div	
14	positive	0,00800		div	

Number positive	8	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:
BS = qualyfast allergen, BIOSIDE
GR = SPECIALfinder Assay, real time PCR, Generon
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

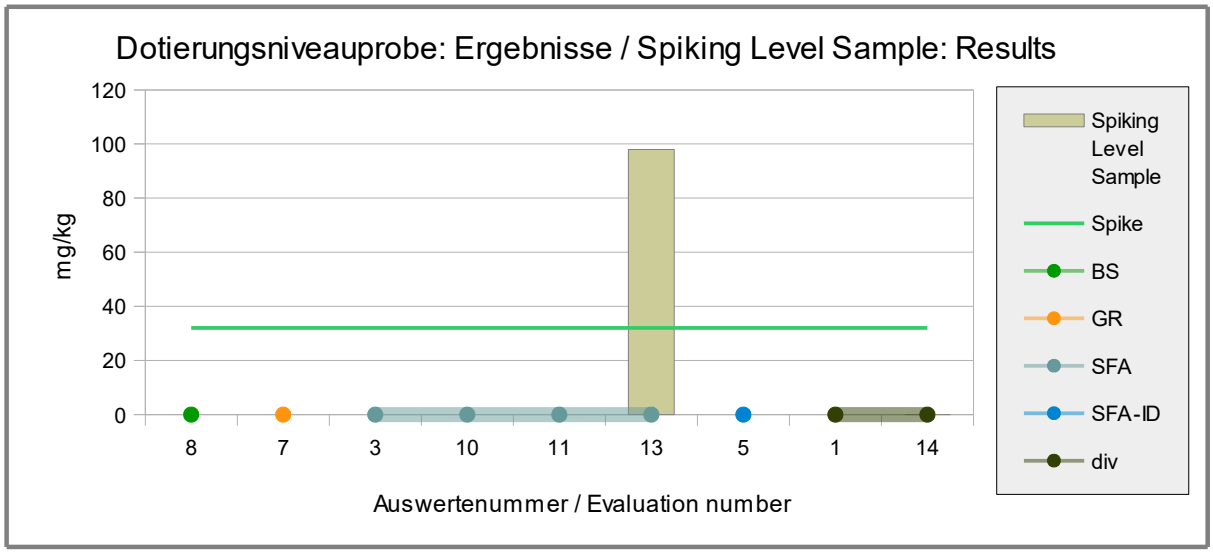


Abb./Fig. 8: PCR Results Pistachio
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Pistachio: 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}]		
8							BS	
7							GR	
3							SFA	
10							SFA	
11							SFA	
13	98,0	306	8,2	74,3	214	4,6	SFA	
5							SFA-ID	
1							div	
14	0,00800	0,02	-4,0				div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BS = qualyfast allergen, BIOSIDE
GR = SPECIALfinder Assay, real time PCR, Generon
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:

No participant obtained a recovery rate in the range of the AOAC requirement of 50-150% with the spiking level sample or with the spiked food matrix sample A by PCR.
The related z-scores are based on the target standard deviation of 25%.

4.1.3 Results other methods: Pistachio

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		
15	negative		negative		-	NGS	no positive sample identified

	Sample A		Sample B	
Number positive	0		0	
Number negative	1		1	
Percent positive	0		0	
Percent negative	100		100	
Consensus value	-		-	

Method:
NGS = Next Generation Sequencing

Comments:
A negative result was obtained for both PT samples A and B. The spiking of sample A was not detected.

Qualitative valuation of results: Spiking Level Sample

Evaluation number	Pistachio	Pistachio	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
15	positive			NGS	

Number positive	1	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	-	

Method:
NGS = Next Generation Sequencing

Comment:
A positive result was obtained for the spiking level sample.

Quantitative evaluation other results: Sample A and Spiking Level Sample

Since no quantitative results were available, no quantitative evaluation was carried out.

4.2 Proficiency Test Mollusks

4.2.1 ELISA Results: Mollusks (as total protein)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
2	positive	24,5	negative	<1	2/2 (100%)	3M	
12	positive	25,0	negative	<LOD	2/2 (100%)	AQ	result converted °
16	positive	29,4	negative	<7,34	2/2 (100%)	AQ	result converted °
1	positive	<22,0	negative	<22,0	2/2 (100%)	SP	result converted °
4	positive	36,7	negative	0	2/2 (100%)	SP	result converted °
17	positive	52,9	negative	<LOQ	2/2 (100%)	SP	result converted °

° calculation see p. 19

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

Methods:
3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative evaluation of ELISA-results: Sample A

Evaluation number	Mollusks protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	24,5	-0,67	3M	
12	25,0	-0,60	AQ	result converted °
16	29,4	0,00	AQ	result converted °
1	<22,0		SP	result converted °
4	36,7	1,0	SP	result converted °
17	52,9	3,2	SP	result converted °

° calculation see p. 19

Methods:
3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
SP = SensiSpec ELISA Kit, Eurofins

Comment:
A kernel density estimation was not carried out due to the number of < 8 results.

Characteristics: Quantitative evaluation ELISA Mollusks (as protein)

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	33,7
Robust Mean	33,7
Median (X_{pt})	**29,4**
Robust standard deviation (S*)	**13,4**
Target range:	
Target standard deviation σ_{pt}	**7,34**
lower limit of target range	**14,7**
upper limit of target range	**44,1**
Quotient S/σ_{pt}*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*7,48*
Results in the target range	*4*
Percent in the target range	*80*

Assigned value (Xpt): Median (see 3.1)

Comments to the statistical characteristics and assigned values:

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 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 median of the evaluation was 56% of the spiking level of mollusks protein to sample A and thus within the range of the recommendations for the applied methods (see 3.4.3 and p.39 "Recovery rates with z-scores ELISA for Mollusks (as protein)").

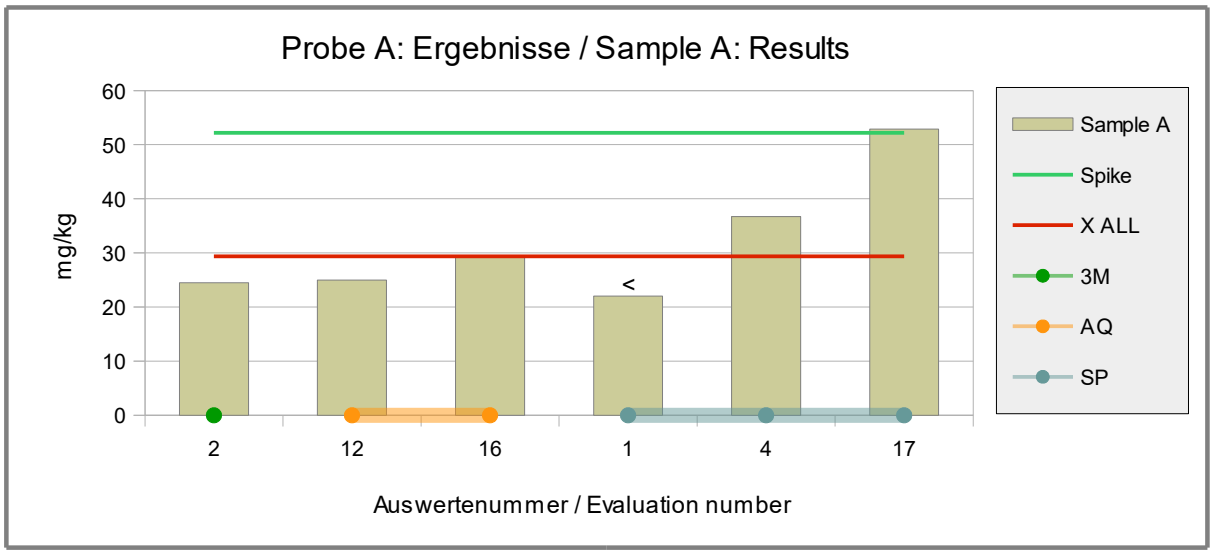


Abb./Fig. 9: ELISA Results Mollusks (as protein)
green line = Spiking level (Spike)
red line = median of all results
round symbols = Applied methods (see legend)

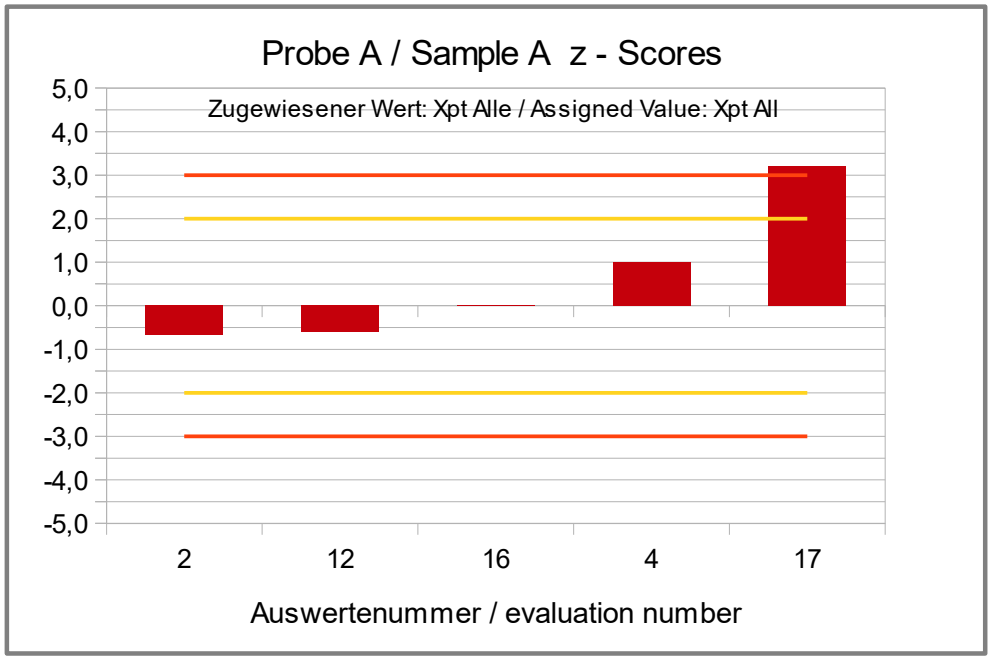


Abb./Fig. 10:
z-Scores (ELISA Results as mollusks protein)
Assigned value: median of all results

Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Mollusks protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	35,9	-0,53	3M	
12	40,4	-0,09	AQ	result converted°
16	36,7	-0,45	AQ	result converted°
1	23,5	-1,7	SP	result converted°
4	47,0	0,55	SP	result converted°
17	88,1	4,5	SP	result converted°

° calculation see p. 19

Methods:

3M = 3M Protein ELISA Kit

AQ = AgraQuant, RomerLabs

SP = SensiSpec ELISA Kit, Eurofins

Comment:

A kernel density estimation was not carried out due to the number of < 8 results.

Characteristics: Quantitative evaluation ELISA Mollusks (as protein)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	6
Number of outliers	0
Mean	45,3
Median	38,6
Robust Mean (X_{pt})	**41,4**
Robust standard deviation (S*)	**15,6**
Target range:	
Target standard deviation σ_{pt}	**10,3**
lower limit of target range	**20,7**
upper limit of target range	**62,0**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*7,96*
Results in the target range	*5*
Percent in the target range	*83*

Comments to the statistical characteristics and assigned values:

The distribution of the results for all methods showed a normal variability. 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 (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 76% of the spiking level of mollusks protein to the spiking level sample and was thus within the range of the recommendations for the applied methods (s. 3.4.3 and p.39 "Recovery rates with z-Scores ELISA for Mollusks (as protein)").

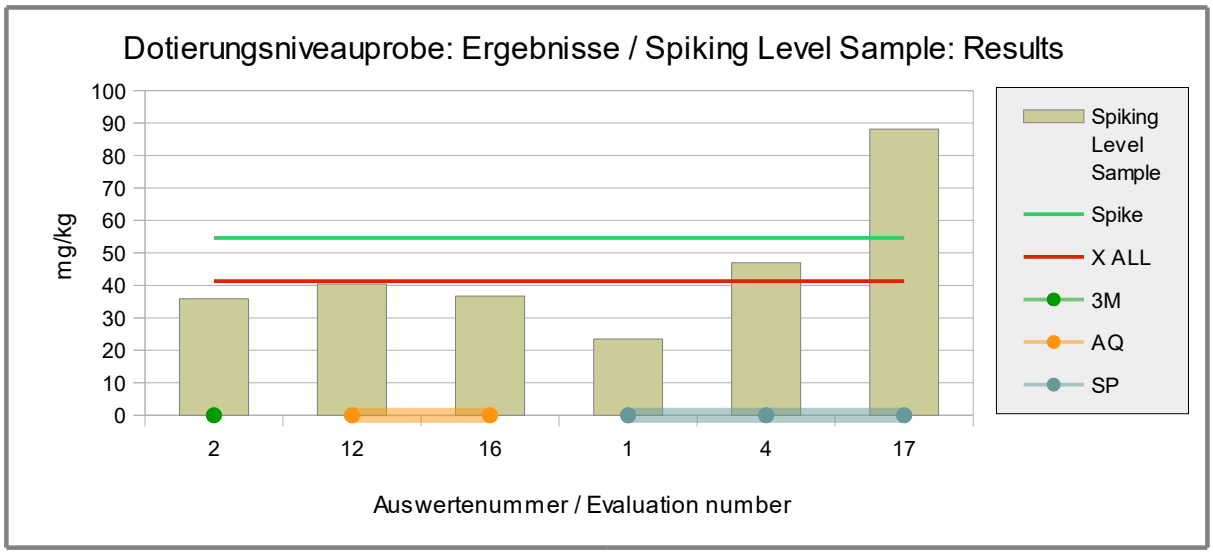


Abb./Fig. 11: ELISA Results Mollusks (as protein)
green line = Spiking level (Spike)
red line = robust mean of all results
round symbols = Applied methods (see legend)

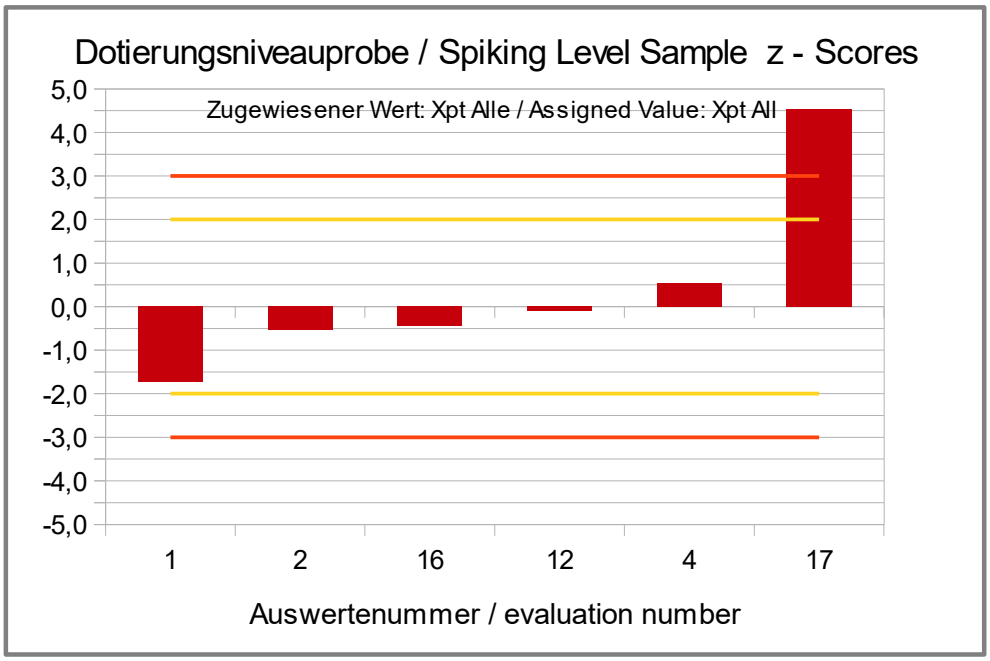


Abb./Fig. 12:
z-Scores (ELISA Results as mollusks protein)
Assigned value: robust mean of all results

Recovery Rates with z-Scores ELISA for Mollusks (as protein): 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}]		
2	35,9	**65,7**	-1,4	24,5	46,9	-2,1	3M	
12	40,4	**74,0**	-1,0	25,0	47,8	-2,1	AQ	result converted °
16	36,7	**67,2**	-1,3	29,4	**56,3**	-1,7	AQ	result converted °
1	23,5	43,0	-2,3	<22,0			SP	result converted °
4	47,0	**86,1**	-0,56	36,7	**70,3**	-1,2	SP	result converted °
17	88,1	161	2,5	52,9	**101**	0,05	SP	result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	4	Number in RA	3
Percent in RA	67	Percent in RA	60

Methods:
3M = 3M Protein ELISA Kit
AQ = AgraQuant, RomerLabs
SP = SensiSpec ELISA Kit, Eurofins

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
67% (4) of the participants 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 A, 60% (3) of the recovery rates were within this range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

Note: With respect to the recovery rates of ELISA methods for the determination of mollusks, special conversion factors for certain mollusk species must be taken into account for some methods (methods: AQ, SP). In this section, for the determination of yesso scallops which are present in the PT samples, the conversion factor for raw scallops was used as the best approximation since there is no factor for yesso scallops (see p.19).

4.2.2 ELISA-Results: Mollusks (as tropomyosin)

Quantitative evaluation of ELISA-results: Sample A

The following evaluation was purely informative due to the low number of results.

Evaluation number	Mollusks tropomyosin	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
12	0,0340	-0,98	AQ	
16	0,0400	-0,44	AQ	
1	<0,03		SP	
4	0,0500	0,44	SP	
17	0,0720	2,4	SP	

Methods:
AQ = AgraQuant, RomerLabs
SP = SensiSpec ELISA Kit, Eurofins

Comment:
A kernel density estimation was not carried out due to the number of < 8 results.

Characteristics: Quantitative evaluation ELISA Mollusks (as tropomyosin)

Sample A

The following evaluation was purely informative.

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	4
Number of outliers	0
Mean	0,0490
Robust Mean	0,0490
Median (X_{pt})	**0,0450**
Robust standard deviation (S*)	**0,0189**
Target range:	
Target standard deviation σ_{pt}	**0,0113**
lower limit of target range	**0,0225**
upper limit of target range	**0,0675**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*0,0118*
Results in the target range	*3*
Percent in the target range	*75*

Assigned value (Xpt): Median (see 3.1)

Comments to the statistical characteristics and assigned values:

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 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 tropomyosin content in sample A is unknown, so that no recovery rate can be given.

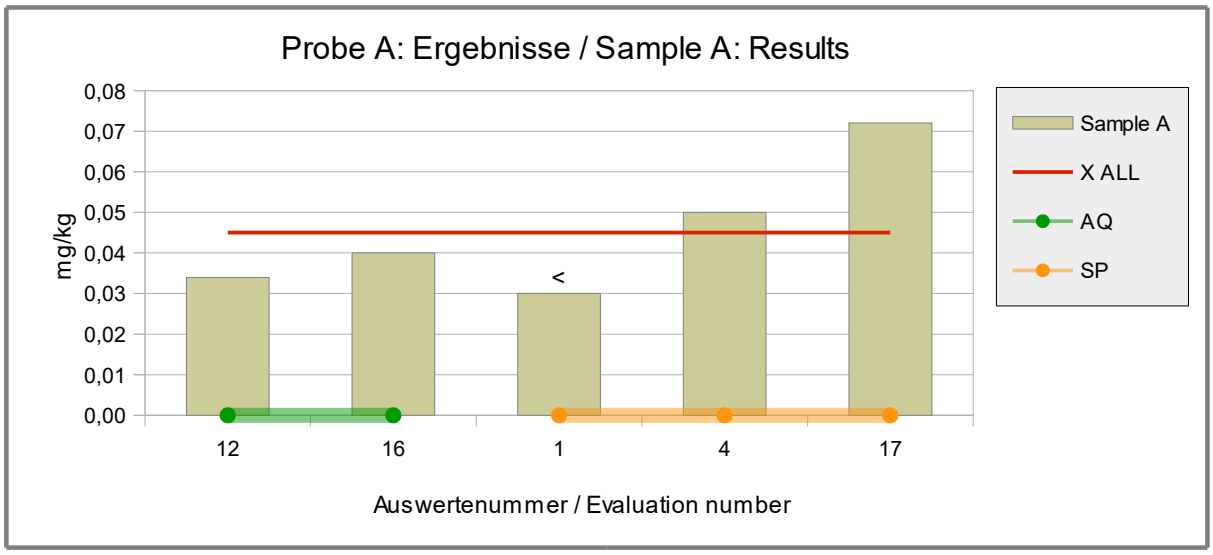


Abb./Fig. 13: ELISA Results Mollusks (as tropomyosin)
red line = median of all results
round symbols = Applied methods (see legend)

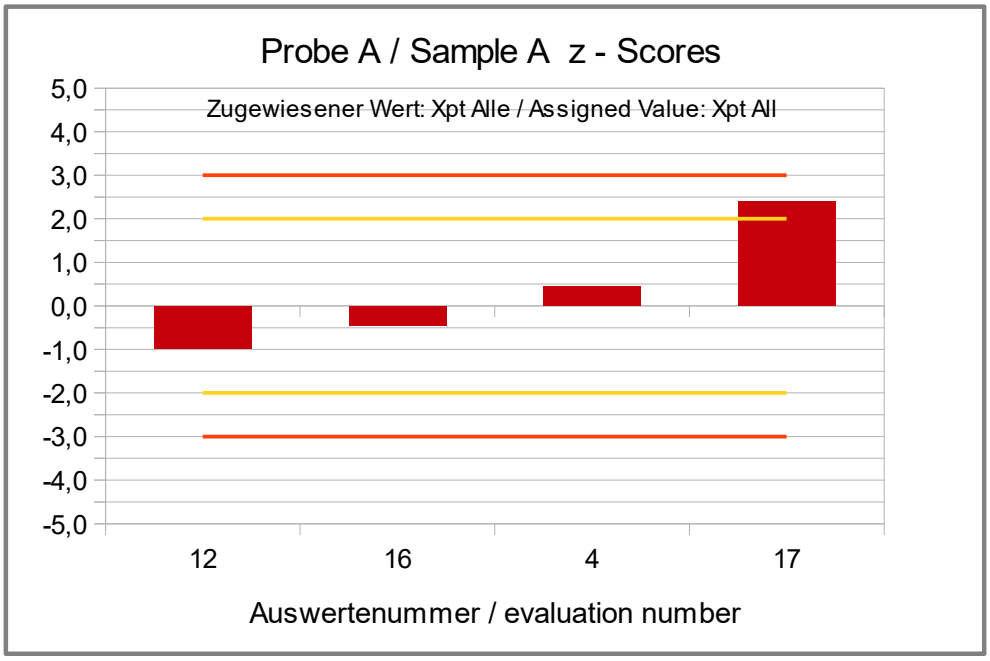


Abb./Fig. 14:
z-Scores informative (ELISA Results as mollusks tropomyosin)
Assigned value: median of all results

Quantitative evaluation of ELISA-results: Spiking Level Sample

Evaluation number	Mollusks tropomyosin	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
12	0,0550	0,00	AQ	
16	0,0500	-0,36	AQ	
1	0,0320	-1,7	SP	
4	0,0640	0,65	SP	
17	0,120	4,7	SP	

Methods:
AQ = AgraQuant, RomerLabs
SP = SensiSpec ELISA Kit, Eurofins

Comment:
A kernel density estimation was not carried out due to the number of < 8 results.

Characteristics: Quantitative evaluation ELISA Mollusks (as tropomyosin)

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	0,0642
Robust Mean	0,0617
Median (Xpt)	**0,0550**
Robust standard deviation (S*)	**0,0319**
Target range:	
Target standard deviation σ_{pt}	**0,0138**
lower limit of target range	**0,0275**
upper limit of target range	**0,0825**
Quotient S/σ_{pt}*	*2,3*
Standard uncertainty $U_{(Xpt)}$	*0,0179*
Results in the target range	*4*
Percent in the target range	*80*

Assigned value (Xpt): Median (see 3.1)

Comments to the statistical characteristics and assigned values:

The distribution of the results for all methods showed an increased variability. The quotient S/σ_{pt} was above 2,0. The robust standard deviation is in the upper 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 tropomyosin content in the spiking level sample is unknown, so that no recovery rate can be given.

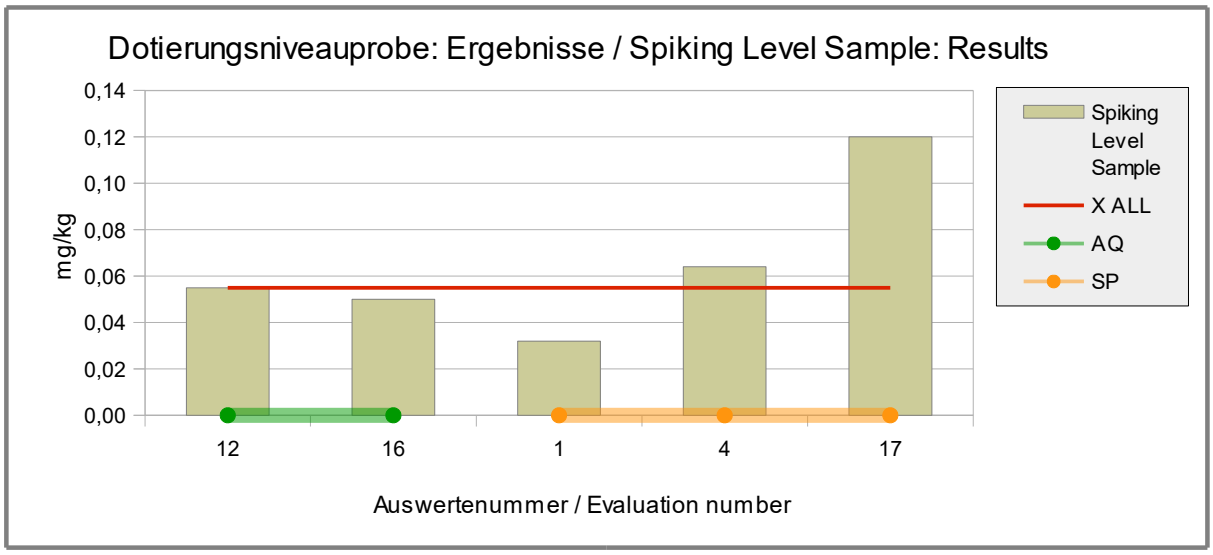


Abb./Fig. 15: ELISA Results Mollusks (as tropomyosin)
red line = median of all results
round symbols = Applied methods (see legend)

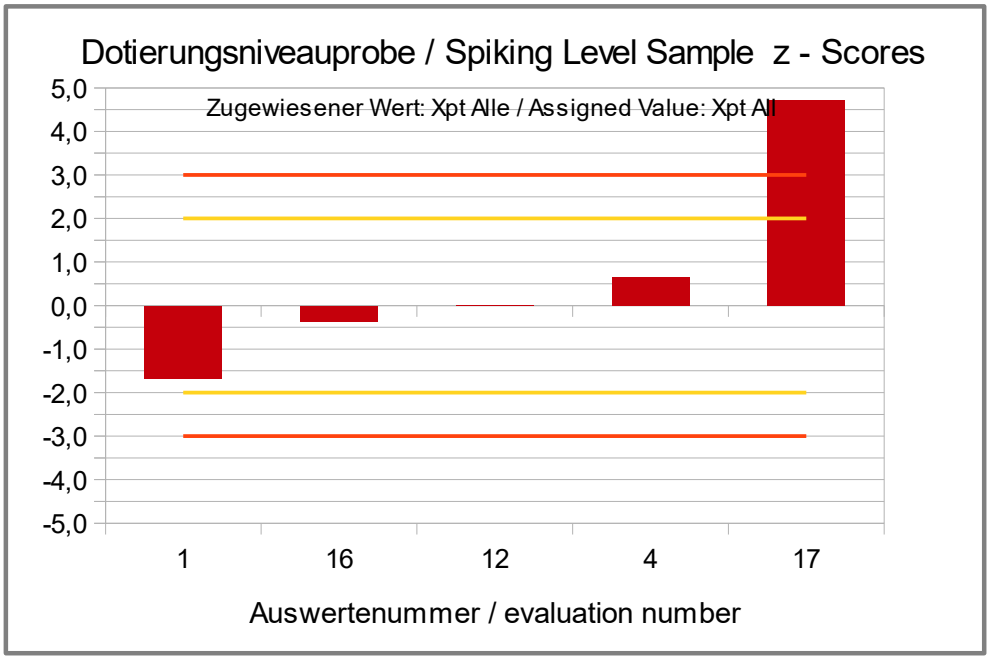


Abb./Fig. 16:
z-Scores (ELISA Results as mollusks tropomyosin)
Assigned value: median of all results

4.2.3 PCR Results: Mollusks

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		
3	positive		negative		2/2 (100%)	SFA	
11	positive		negative		2/2 (100%)	SFA	
13	positive	106	negative	<1	2/2 (100%)	SFA	
5	positive		negative		2/2 (100%)	SFA-ID	
14	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	5		0	
Number negative	0		5	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

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

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

Quantitative evaluation PCR: Sample A

The quantitative results were not evaluated because too few single results were available.

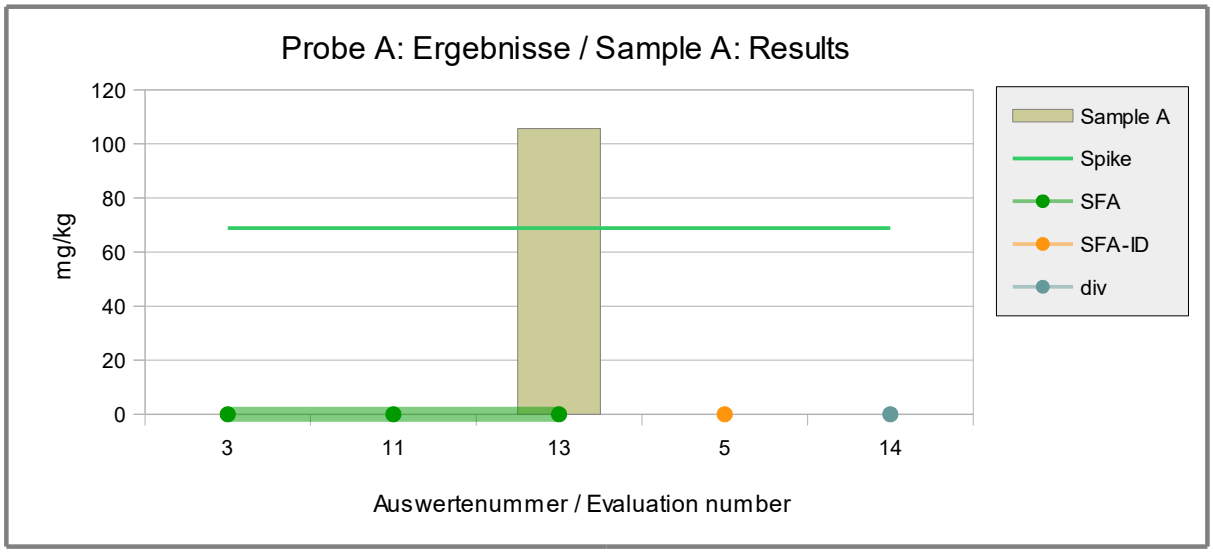


Abb./Fig. 17: PCR Results Mollusks
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Quantitative evaluation PCR: Spiking Level Sample

The quantitative results were not evaluated because too few single results were available.

Evaluation number	Mollusks	Mollusks	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
3	positive			SFA	
11	positive			SFA	
13	positive	150		SFA	
5	positive			SFA-ID	
14	positive	0,0800		div	

Number positive	5	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:
SFA = Sure Food ALLERGEN, 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.

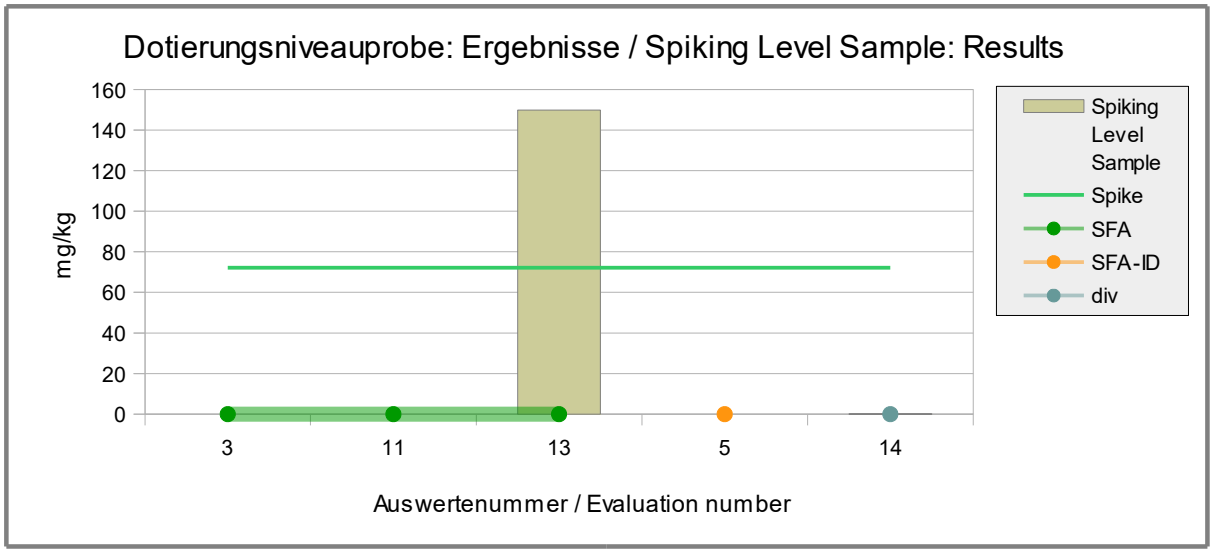


Abb./Fig. 18: PCR Results Mollusks
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Mollusks: 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	
11							SFA	
13	150	208	4,3	106	153	2,1	SFA	
5							SFA-ID	
14	0,0800	0,11	-4,0				div	

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

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

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
No participant obtained a recovery rate in the range of the AOAC requirement of 50-150% with the spiking level sample or with the spiked food matrix sample A by PCR.
The related z-scores are based on the target standard deviation of 25%.

4.2.4 Results other methods: Mollusks

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		
15	positive		negative		-	NGS	

	Sample A		Sample B	
Number positive	1		0	
Number negative	0		1	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	-		-	

Method:
NGS = Next Generation Sequencing

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

Qualitative valuation of results: Spiking Level Sample

Evaluation number	Mollusks	Mollusks	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
15	positive			NGS	

Number positive	1	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	-	

Method:
NGS = Next Generation Sequencing

Comment:
A positive result was obtained for the spiking level sample.

Quantitative evaluation other results: Sample A and Spiking Level Sample

Since no quantitative results were available, no quantitative evaluation was carried out.

4.3 Proficiency Test Celery

4.3.1 ELISA Results: Celery

No ELISA results were submitted for the parameter celery.

4.3.2 PCR Results: Celery

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	positive		negative		2/2 (100%)	ASU	
6	positive		negative		2/2 (100%)	ASU	
18	positive	20,3	negative	<LOD	2/2 (100%)	FP	
7	positive		negative		2/2 (100%)	GR	
8	negative		negative		1/2 (50%)	GR	no positive sample identified
3	positive		positive		1/2 (50%)	SFA	
9	positive		negative		2/2 (100%)	SFA	
11	positive		negative		2/2 (100%)	SFA	
13	positive	90,0	negative	<1	2/2 (100%)	SFA	
10	positive		negative		2/2 (100%)	SFA-4p	
5	positive		negative		2/2 (100%)	SFA-ID	
14	positive		negative		2/2 (100%)	div	
16	negative		negative		1/2 (50%)	div	no positive sample identified

	Sample A		Sample B	
Number positive	11		1	
Number negative	2		12	
Percent positive	85		8	
Percent negative	15		92	
Consensus value	positive		negative	

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GR = SPECIALfinder Assay, real time PCR, Generon
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:
The consensus values are in qualitative agreement with the spiking of sample A. Two negative results were obtained for sample A.

Quantitative evaluation PCR: Sample A

The quantitative results were not evaluated because too few single results were available.

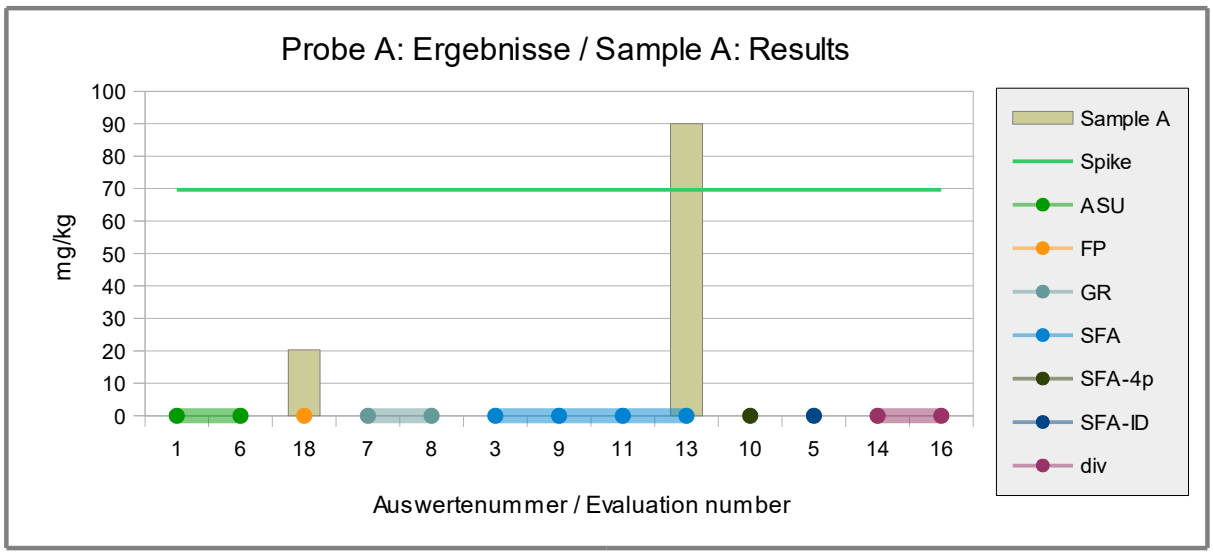


Abb./Fig. 19: PCR Results Celery
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Quantitative evaluation PCR: Spiking Level Sample

The quantitative results were not evaluated because too few single results were available.

Evaluation number	Celery	Celery	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positive			ASU	
6	positive			ASU	
18	positive	24,7		FP	
7	-			GR	
8	positive			GR	
3	positive			SFA	
9	positive			SFA	
11	positive			SFA	
13	positive	143		SFA	
10	positive			SFA-4p	
5	positive			SFA-ID	
14	positive	0,00800		div	
16	positive			div	

	Sample A	
Number positive	12	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GR = SPECIALfinder Assay, real time PCR, Generon
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.

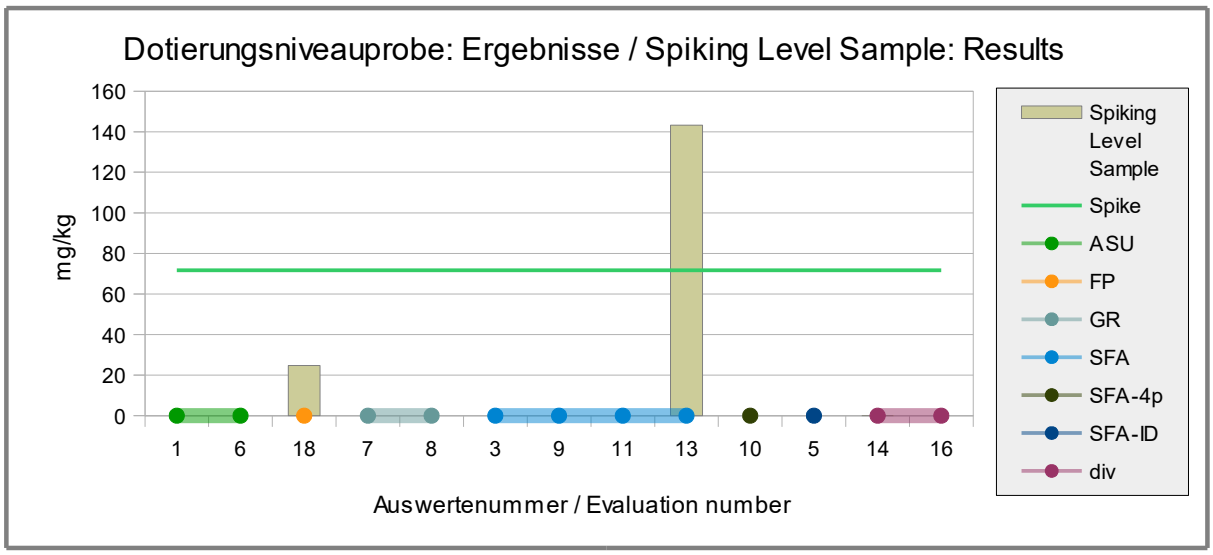


Abb./Fig. 20: PCR Results Celery
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Celery: 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}]		
1							ASU	
6							ASU	
18	24,7	34,4	-2,6	20,3	29,2	-2,8	FP	
7							GR	
8							GR	
3							SFA	
9							SFA	
11							SFA	
13	143	200	4,0	90,0	**129**	1,2	SFA	
10							SFA-4p	
5							SFA-ID	
14	0,00800	0,01	-4,0				div	
16							div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GR = SPECIALfinder Assay, real time PCR, Generon
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:

No participant obtained a recovery rate in the range of the AOAC requirement of 50-150% with the spiking level sample by PCR. For the spiked food matrix sample A, one (50%) of the recory rates was within this range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

Note: It should be noted that the PT samples contain celery leaves, while the participants probably reported the results as celery seed.

4.3.3 Results other methods: Celery

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		
15	negative		negative		-	NGS	no positive sample identified

	Sample A		Sample B	
Number positive	0		0	
Number negative	1		1	
Percent positive	0		0	
Percent negative	100		100	
Consensus value	-		-	

Method:
NGS = Next Generation Sequencing

Comments:
A negative result was obtained for both PT samples A and B. The spiking of sample A was not detected.

Qualitative valuation of results: Spiking Level Sample

Evaluation number	Celery	Celery	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
15	positive			NGS	

Number positive	1	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	-	

Method:
NGS = Next Generation Sequencing

Comment:
A positive result was obtained for the spiking level sample.

Quantitative evaluation other results: Sample A and Spiking Level Sample

Since no quantitative results were available, no quantitative evaluation was carried out.

4.4 Participant z-Scores: overview table

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

Evaluation number	ELISA Pistachio **Xpt** (div. Methods)		ELISA Mollusks (Total Protein) **Xpt** (div. Methods)		ELISA Mollusks (Tropomyosin) **Xpt** (div. Methods)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A*	Spiking Level Sample
1				-1,7		-1,7
2	-2,9	-3,1	-0,67	-0,53		
3						
4			1,0	0,55	0,44	0,65
5						
6	-0,02	-0,18				
7						
8						
9						
10						
11						
12	2,0	4,3	-0,60	-0,09	-0,98	0,00
13	0,08	-0,10				
14						
15						
16			0,00	-0,45	-0,44	-0,36
17	1,0	1,2	3,2	4,5	2,4	4,7
18	0,58	-0,32				
19	-1,2	-0,75				

** z-Scores purely informative*

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 Pistachio Xpt (Spike)		ELISA Mollusks (Total Protein) Xpt (Spike)		PCR Pistachio Xpt (Spike)		PCR Mollusks Xpt (Spike)		PCR Celery Xpt (Spike)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1				-2,3						
2	-1,6	-2,0	-2,1	-1,4						
3										
4			-1,2	-0,56						
5										
6	4,7	4,2								
7										
8										
9										
10	37	35								
11										
12	9,0	14	-2,1	-1,0						
13	4,9	4,4			4,6	8,2	2,1	4,3	1,2	4,0
14						-4,0		-4,0		-4,0
15										
16			-1,7	-1,3						
17	7,0	7,1	0,05	2,5						
18	6,0	3,9							-2,8	-2,6
19	2,2	3,0								

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: Pistachio

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
3M	2	03.12.21	positive	4,56	negative	<1	positive	3,5		1		Pistachio protein	3M Pistachio Protein ELISA Kit E96PST
AQ	6	28.12.21	-	75,61	-	< LOQ	-	65,82		1		Pistachio, total	AgraQuant ELISA Pistachio , RomerLabs
AQ	12	07.01.22	positive	113	negative	<LOD	positive	143	0,04	0,12		whole pistachio	AgraQuant, RomerLabs
AQ	16	07.01.22	negative	<1	negative	<1	negative	<1		1		Pistachio, total	AgraQuant ELISA Pistachio COKAL2748, RomerLabs
AQ-P	10	04.01.22	positive	77,95	negative	< 1	positive	68,06				Pistachio protein	AgraQuant Plus ELISA Pistachio COKAL2748F, RomerLabs
BC	13	15.12.21	positive	77,44	negative	<1	positive	67,25	1	1	27,7	Pistachio, total	BioCheck ELISA Pistachio-Check
BF	5		positive	>40	negative	< 1	positive	>40		1		Pistachio protein	MonoTrace Pistachio ELISA kit, BioFront Technologies
BF	19	22/2	positive	54	positive	ND	positive	56	0,12	1		Pistachio, total	MonoTrace Pistachio ELISA kit, BioFront Technologies
IL	14		positive		negative		positive		1	2	21	Pistachio protein	Immunolab Pistachio ELISA
IL	18	28.12.21	positive	87	negative	<LOQ	positive	63,5	x	1	18,3	Pistachio, total	Immunolab Pistachio ELISA
SP	17	17.12.21	positive	95	negative	<LOQ	positive	89	0,07	1		Pistachio, total	Eurofins SensiSpec Pistachio ELISA Kit

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

Meth. Abr.	Evaluation number	Specifity	Remarks on 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 B: 115%
AQ	6		according to test instructions	Yes	
AQ	12				
AQ	16				
AQ-P	10			no	
BC	13	As Per Kit Instrcutions	As Per Kit Instrcutions	Yes	
BF	5			yes	
BF	19	Monoclonal Antibody	1:10 extraction ratio, 10 minutes @ 60C	no	ND = not detected
IL	14				
IL	18		Samples dilluted 1:3, extraction reagent added to A,B due to spices	Yes	Sample B was >LOD but <LOQ. According to method specs values found < LOQ should be mentioned as negative. However sample B found to be >LOD (0,55-1,3 ppm)
SP	17				

5.1.2 ELISA: Mollusks

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
3M	2	03.12.21	positive	24,47	negative	<1	positive	35,86		1		Mollusks protein	3M Mollusk Protein ELISA Kit E96MOL
AQ	12	07.01.22	positive	0,034	negative	<LOD	positive	0,055	0,0007	0,002		mollusk tropomyosin	AgraQuant, RomerLabs
AQ	16	07.01.22	positive	0,04	negative	<0.01	negative	0,05		0,01		Tropomyosin	AgraQuant ELISA Mollusk RomerLabs
SP	1	22.11.	positive	<0,03	negative	<0,03	positive	0,032	0,01	0,03		Tropomyosin	Eurofins SensiSpec Molluscs (Tropomyosin) ELISA Kit
SP	4	2021-12-17	positive	0,05	negative	0	positive	0,064	0,0017	0,011	-	Tropomyosin	Eurofins SensiSpec Molluscs (Tropomyosin) ELISA Kit
SP	17	17.12.21	positive	0,072	negative	<LOQ	positive	0,12	0,002	0,01		Tropomyosin	Eurofins SensiSpec Molluscs (Tropomyosin) ELISA Kit

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

Meth. Abr.	Evaluation number	Specifity	Remarks on 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 B: 106%
AQ	12				
AQ	16				
SP	1	detects mollusk tropomyosin	according to manufacturer information	yes	HU0030015/HU0030039; Sample A: 0,024mg/kg
SP	4	Tropomyosin	1. Extraction solution supply by test kit/ time; 15 min/ temparature; 60 C 2. ELISA time; 60 min/ incubation temparature 20-25 C	No	
SP	17				

5.1.3 PCR: Pistachio

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
BS	8	03.01.22	positive		negative		positive		5			Please select!	qualyfast allergen, BIOSIDE
GR	7	22.11.21	positive		negative		-		100			Please select!	SPECIALfinder Assay, real time PCR, Generon
SFA	3		positive		negative		positive		0,4			Food	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10	04.01.22	positive		negative		positive					DNA-Pistachio	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		positive		negative		positive		0,4			DNA-Pistachio	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	13	17.11.21	positive	74,26	negative	<1	positive	98	1	1	45	Pistachio, total	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	5		positive		negative		positive		0,4			DNA-Pistachio	Sure Food Allergen ID, R-Biopharm / Congen
div	1	12.11.	positive		negative		positive		0,5			DNA-Pistachio	Internal method
div	14		positive		negative		positive	0,008	0,08			Please select!	Selection PCR-Methods

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

Meth. Abr.	Evaluation number	Specifity	Remarks on 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 / cycles	yes/no	
BS	8			yes	
GR	7			yes	
SFA	3				
SFA	10		SureFood Prep Advanced Protocol 1	no	
SFA	11		real time PCR	no	
SFA	13	As Per Kit Instrcutions	As Per Kit Instrcutions	Yes	
SFA-ID	5			yes	
div	1		CTAB / Proteinase K / Rnase A / Promega Maxw ell / realtime PCR	Yes	
div	14				

5.1.4 PCR: Mollusks

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
SFA	3		positive		negative		positive		0,4			Food	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		positive		negative		positive		0,4			DNA-Mollusks	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	13	17.11.21	positive	105,64	negative	<1	positive	149,77	1	1	48,44	Mollusks, fresh	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	5		positive		negative		positive		0,4			DNA-Mollusks	Sure Food Allergen ID, R-Biopharm / Congen
div	14		positive		negative		positive	0,08	/			Please select!	Selection PCR-Methods

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

Meth. Abr.	Evaluation number	Specifity	Remarks on 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 / cycles	yes/no	
SFA	3				
SFA	11		real time PCR	no	
SFA	13	As Per Kit Instrcutions	As Per Kit Instrcutions	Yes	
SFA-ID	5			yes	
div	14				

5.1.5 PCR: Celery

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
ASU	1	12.11.	positive		negative		positive		10			Celery-DNA	ASU §64 Methode/method
ASU	6	24.11.21	positive		negative		positive					Celery-DNA	ASU §64 Methode/method
FP	18	30.12.21	positive	20,3	negative	<LOD	positive	24,7	0,1	0,8		Celery-DNA	foodproof Detection Kit, BIOTECON Diagnostics
GR	7	22.11.21	positive		negative		-		100			Please select!	SPECIALfinder Assay, real time PCR, Generon
GR	8	04.01.22	negative		negative		positive		0,5			Please select!	SPECIALfinder Assay, real time PCR, Generon
SFA	3		positive		positive		positive		0,4			Food	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9	24.12.22	positive		negative		positive		0,4			Celery	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		positive		negative		positive		0,4			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	13	17.11.21	positive	89,96	negative	<1	positive	143,26	1	1	45,94	Celery seed, dried	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	10	04.01.22	positive		negative		positive					DNA-Pistachio	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	5		positive		negative		positive		0,4			Celery-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	14		positive		negative		positive	0,008	0,08			Please select!	Selection PCR-Methods
div	16	07.01.22	negative		negative		positive		10			Celery	

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

Meth. Abr.	Evaluation number	Specifity	Remarks on 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 / cycles	yes/no	
ASU	1		CTAB / Proteinase K / Rnase A / Promega Maxwell / realtime PCR	Yes	§ 64 LFGB L 08.00-56
ASU	6	Mannitol dehydrogenase protein	Spiking Level Sample: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles Sample A+B: Dneasy Mericon Food-Kit/ Proteinase K/ Real Time PCR/ 45 cycles	Yes	
FP	18		st curve 301221		
GR	7			yes	
GR	8			yes	
SFA	3				
SFA	9	DNA		yes	
SFA	11		real time PCR	no	
SFA	13	As Per Kit Instrcutions	As Per Kit Instrcutions	Yes	
SFA-4p	10		SureFood Prep Advanced Protocol 1	no	
SFA-ID	5			yes	
div	14				
div	16		in house method		

5.1.6 Results other methods: Pistachio

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
NGS	15		negative		negative		positive						NGS

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

Meth. Abr.	Evaluation number	Specifity	Remarks on the method (extraction and determination)	Method accredited ISO/IEC 17025	Further Remarks
NGS	15			Yes	

5.1.7 Results other methods: Mollusks

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
NGS	15		positive		negative		positive						NGS

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

Meth. Abr.	Evaluation number	Specifity	Remarks on the method (extraction and determination)	Method accredited ISO/IEC 17025	Further Remarks
NGS	15			Yes	

5.1.8 Results other methods: Celery

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%		Test-Kit + Manufacturer
NGS	15		negative		negative		positive						NGS

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

Meth. Abr.	Evaluation number	Specifity	Remarks on the method (extraction and determination)	Method accredited ISO/IEC 17025	Further Remarks
NGS	15			Yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA-ptAL07 Sample A

Weight whole sample	2,60	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	40	16,0
2	5,05	56	22,2
3	5,06	43	17,0
4	4,98	54	21,7
5	5,02	44	17,5
6	4,97	54	21,7
7	4,97	53	21,3
8	4,99	57	22,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	50,1	Particles
Standard deviation	6,79	Particles
χ^2 (CHI-Quadrat)	6,44	
Probability	**49**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	20,0	mg/kg
Standard deviation	2,71	mg/kg
rel. Standard deviaton	13,5	%
Horwitz standard deviation	10,2	%
HorRat-value	**1,3**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA ptAL07 Spiking Level Sample

Weight whole sample	1,00	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	58	23,2
2	4,98	57	22,9
3	5,02	72	28,7
4	5,02	78	31,1
5	5,02	57	22,7
6	5,02	73	29,1
7	5,02	70	27,9
8	4,98	61	24,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,7	Particles
Standard deviation	8,24	Particles
χ^2 (CHI-Quadrat)	7,23	
Probability	**41**	%
Recovery rate	93	%

Normal distribution		
Number of samples	8	
Mean	26,3	mg/kg
Standard deviation	3,29	mg/kg
rel. Standard deviaton	12,5	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,3**	
Recovery rate	93	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL07 - 2021***
PT name	***Allergens VII: Pistachio, Mollusks and Celery in Instant Noodle Soup***
Sample matrix (processing)	***Samples A + B:*** *Broccoli Noodle Soup/ ingredients: Gluten-free noodles (40%), maltodextrin, palm fat, modified starch, glucose syrup, iodized table salt, rice flour, yeast extract, 1.4% broccoli, leek, onions, stabilizer diphosphate, spinach, thickener xanthan gum, garlic, spices, vegetable juice concentrates (carrot, two belly), flavor, tomato extract, sunflower oil, lovage, 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:* *Pistachio, Mollusks and Celery (Protein, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest January 7th 2022***
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
		GREECE
		CANADA
		ITALY
		ITALY
		Germany
		FRANCE
		SPAIN
		THAILAND
		Germany
		Germany
		GREAT BRITAIN
		FRANCE
		ITALY
		GREAT BRITAIN
		ITALY
		PORTUGAL
		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.]

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
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