Proficiency Tests
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Evaluation Report
proficiency test

DLA 07/2018

Allergens VII:

Crustaceae and Coconut

in Instant Product (Tomato sauce powder)

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (12 April 2019) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragter / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 12 April 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is instant sauce powder with addition of potato flour. The basic composition of samples A and B was the same (see table 1). After crushing and sieving (mesh 2,5 mm) the basic mixture was homogenized.
Afterwards the **spiked sample A** was produced as follows:
The spiking materials containing the allergenic ingredients crayfish powder and coconut flour were sieved by a centrifugal mill (mesh 250 µm) and added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 3 additional steps and homogenized in each case until the total quantity had been reached.

For the **spiking level sample,** the allergenic compounds above mentioned (mesh 250µm) were added during a multi-stage addition of potato powder (mesh 500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Tomato sauce powder Ingredients: Tomatoes, starch, sugar, salt, rice flour, palm oil, onions, yeast extract, vegetable oil, spices, acidifier: citric acid, herbs, basil extract Nutrients per 100 g: Fat 7,5 g, Carbohydrates 58 g, Fiber 5,8 g, Protein 8,3 g, Salt 12 g	72,6 g/100 g	76,3 g/100g	-
Potato Flour Nutrients per 100g: Protein 0 g	22,5 g/100 g	23,7 g/100g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	4,6 g/100 g	-	99,8 g/100 g
Crayfish powder *Ingredients*: Louisiana-crayfish-meat *(Procambarus clarkii)*, cooked, dried - as Crayfish, dried* - thereof 79% total protein**	 75,1 mg/kg 59,1 mg/kg	-	 72,6 mg/kg 57,1 mg/kg
Coconat flour: - as Coconut flour* - *thereof 17% total protein***	 460 mg/kg 78,7 mg/kg	-	 445 mg/kg 76,1 mg/kg
further Ingredients: *Maltodextrin, titanium dioxide, silicon dioxide and potassium sorbate*	<0,3 g/100 g	-	<0,2 g/100 g

* Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=6,25 for Crustaceae protein and with F=5,30 for Coconut protein)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples A and the spiking level sample showed a probability of 71% and 97%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17].
This gave a HorRat value of 1,1 and 0,5 respectively. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample A by all ELISA tests for coconut (Immunolab) and crustaceae (Immunolab, Veratox and AgraQuant) (see pages 7-8). 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].

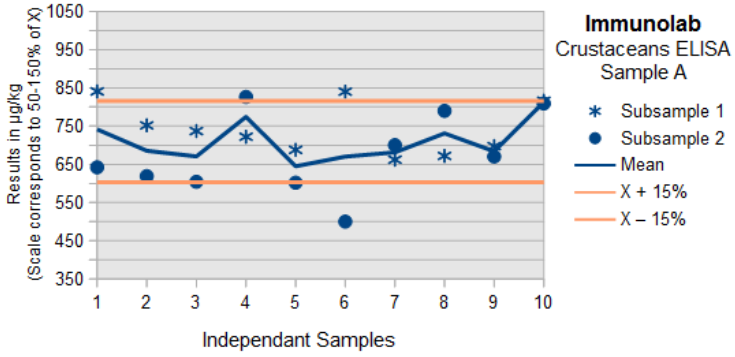
ELISA-Tests: Homogenität Crustaceae / Homogeneity Crustaceae

Immunolab Crustaceans ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomyosin 710 ± 53 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	841	642	741
2	752	619	686
3	737	604	671
4	722	826	774
5	688	602	645
6	840	500	670
7	662	700	681
8	672	790	731
9	698	670	684
10	816	809	813

General average X	710	
SD of sample means Sx	53,3	7,5%
SD within-samples Sw	74,9	10,6%
SD between-samples Ss	< 53,3	< 7,5%

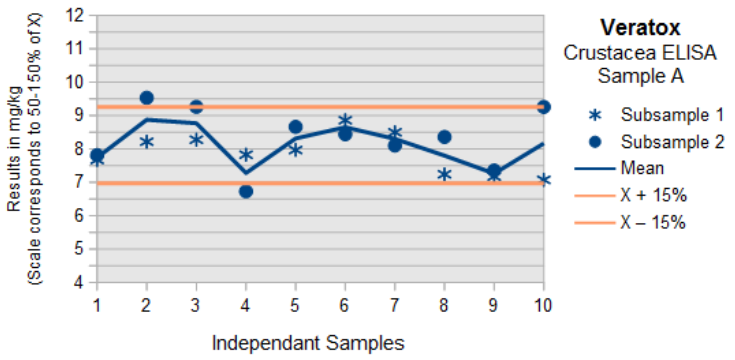


Neogen Veratox ELISA Crustacea

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Crustacea (as dried shrimps) 7,61 ± 0,45 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	7,17	7,31	7,24
2	7,71	9,03	8,37
3	7,77	8,76	8,27
4	7,33	6,23	6,78
5	7,47	8,16	7,81
6	8,36	7,94	8,15
7	8,00	7,60	7,80
8	6,74	7,86	7,30
9	6,67	6,85	6,76
10	6,57	8,76	7,66

General average X	7,61	
SD of sample means Sx	0,580	7,6%
SD within-samples Sw	0,519	6,8%
SD between-samples Ss	0,449	5,9%

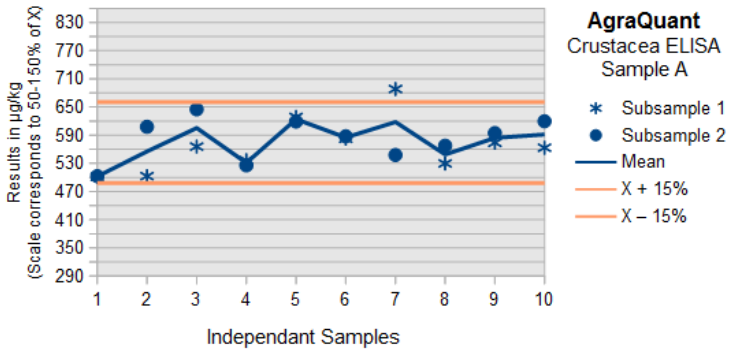


Romerlabs AgraQuant Crustacea

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Tropomosin 575 ± 32 mg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	501	503	502
2	503	608	555
3	566	645	605
4	537	526	531
5	629	619	624
6	582	588	585
7	688	548	618
8	530	568	549
9	574	595	584
10	563	620	591

General average X	575	
SD of sample means Sx	39,4	6,9%
SD within-samples Sw	32,5	5,7%
SD between-samples Ss	31,9	5,6%



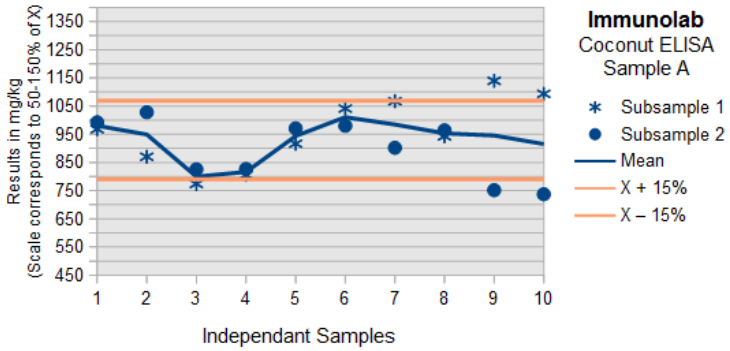
ELISA-Tests: Homogenität Kokosnuss / Homogeneity Coconut

Immunolab Coconut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Coconut, fresh 930 ± 24 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	968	992	980
2	870	1028	949
3	774	826	800
4	806	827	816
5	916	971	944
6	1041	980	1011
7	1067	902	985
8	943	964	953
9	1139	752	946
10	1094	737	915

General average X	930	
SD of sample means Sx	69,5	7,5%
SD within-samples Sw	92,3	9,9%
SD between-samples Ss	23,8	2,6%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,29 (24,3°C) The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 47th week of 2018. The testing method was optional. The tests should be finished at 4th January 2019 the latest.

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

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

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 limit of quantification, specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

Out of 14 participants, 13 participants submitted their results in time. One participant has not submitted any results.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 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**
Almond	Rice cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR multiplex ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR multiplex ASU 18.00-22
Soy	Wheat flour Corn flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soy 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
Soy flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soy 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 information the z-scores below are calculated with a target standard deviation of 25%:

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.

An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty of the assigned value

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(Xpt)}$) 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

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

3.10 Recovery rates: Spiking

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

4. Results

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

The following result sections are structured equally for the allergenic components. First all results of ELISA or PCR methods for a certain parameter are reported for samples A and B (qualitative / possibly quantitative) and afterwards for the spiking level sample (quantitative). The recovery rates of results for the spiking level sample and the spiked sample A or B are reported then.
In the result chapter all quantitative results of the participants are displayed formatted to 3 decimal places. In the documentation, all results are given as they were transmitted by the participants.

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

The **ELISA results** for Crustaceae were evaluated as Crustaceae protein. Depending on the applied test kit, results given as the fresh product have been converted directly into Crustaceae protein (test kit manual: Ridascreen with 20% protein, handbook: Veratox with 22.78% protein) or first in Crustaceae, dried (BioFront). In the second case, a dry weight of 21.8% (experimental value of crayfish raw material) was taken as a basis and then converted to the total protein content using the experimentally determined protein content of 79% in the dry matter (see p.5).
Results given as Crustaceae dry weight (AgraQuant) have also been converted to the total protein content with the experimentally determined protein content of 79% in dry matter (see p.5).

ELISA results, expressed as tropomyosin, have been converted to total protein using the manufacturer's data of 20% tropomyosin in the total protein (AgraQuant, Immunolab, Eurofins).

Results given as coconut fresh (ELISA Immunolab) or indicated as such according to the manufacturer's instructions (coconut = fresh coconut, personal communication) (ELISA Eurofins SensiSpec) have been converted into the dry product coconut flour. This was based on a dry matter of 55.2% (food composition and nutrition tables Souci, Fachmann, Kraut).

The **PCR result** for crustacea, fresh was converted with a dry weight of 21.8% (experimental value of crayfish raw material) into crustacea, dried.

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

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

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

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

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

Characteristics	**All Results** *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ i}}$
Number of results		
Number of outliers		
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 $U_{(Xpt)}$		
Number of results in target range		
Percent in target range		

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

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

4.1 Proficiency Test Crustaceae

4.1.1 ELISA Results: Crustaceae (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	0,390	negative	<0,02	2/2 (100%)	AQ	
3	positive	0,956	negative	<0,08	2/2 (100%)	AQ	Result converted °
6	positive	0,351	negative	<0,02	2/2 (100%)	AQ	
9	positive	0,900	negative	<0,1	2/2 (100%)	AQ	
1	positive	6,04	negative	0	2/2 (100%)	BF	Result converted °
7	positive	3,35	negative	<0,1	2/2 (100%)	EF	Result converted °
10	negative	<0,25	negative	<0,25	1/2 (50%)	ES	no positive sample detected, Result converted °
13	positive	3,64	negative	0	2/2 (100%)	IL	Result converted °
4	positive	6,78	negative	<4	2/2 (100%)	RS-F	Result converted °
5	negative		negative		1/2 (50%)	RS-F	no positive sample detected
11	positive	1,75	negative	<0,57	2/2 (100%)	VT	Result converted °

° calculation see p. 19

	Sample A		Sample B	
Number positive	9		0	
Number negative	2		11	
Percent positive	82		0	
Percent negative	18		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. Two negative results with methods ES and RS-F were obtained for sample A.

Quantitative valuation of ELISA-results: Sample A

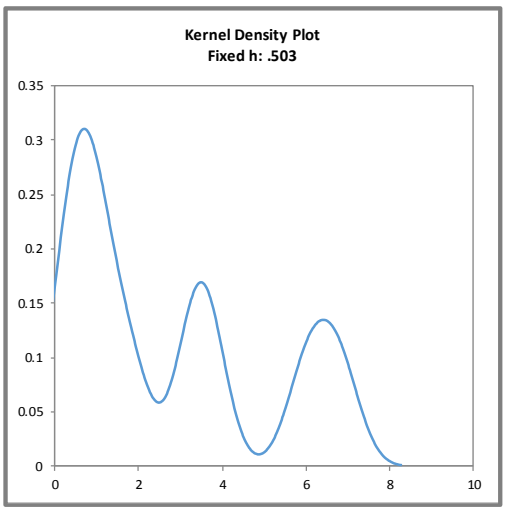


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows 3 maxima for the results obtained from 4 values (method AQ), 3 values (methods EF, IL and VT) and 2 values (methods BF and RS-F) (see Figure 1).

Characteristics: Quantitative evaluation ELISA: Crustaceae (as total protein)

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	9
Number of outliers	
Mean	2,68
Median	1,75
Robust Mean (X)	2,68
Robust standard deviation (S*)	2,75
Target range:	
Target standard deviation σ_{pt}	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Results in the target range	
Percent in the target range	

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a maximum at values of 0.35 to 0.9 mg/kg (4 results method AQ), as well as two other smaller maxima (other methods). A joint evaluation of the results of different methods was not possible. Due to the small number of results, no evaluation was made for the single methods.
The individual quantitative results were evaluated in comparison to the spiking level for information (see "Recovery rates for Crustaceae (as total protein)" p.28).

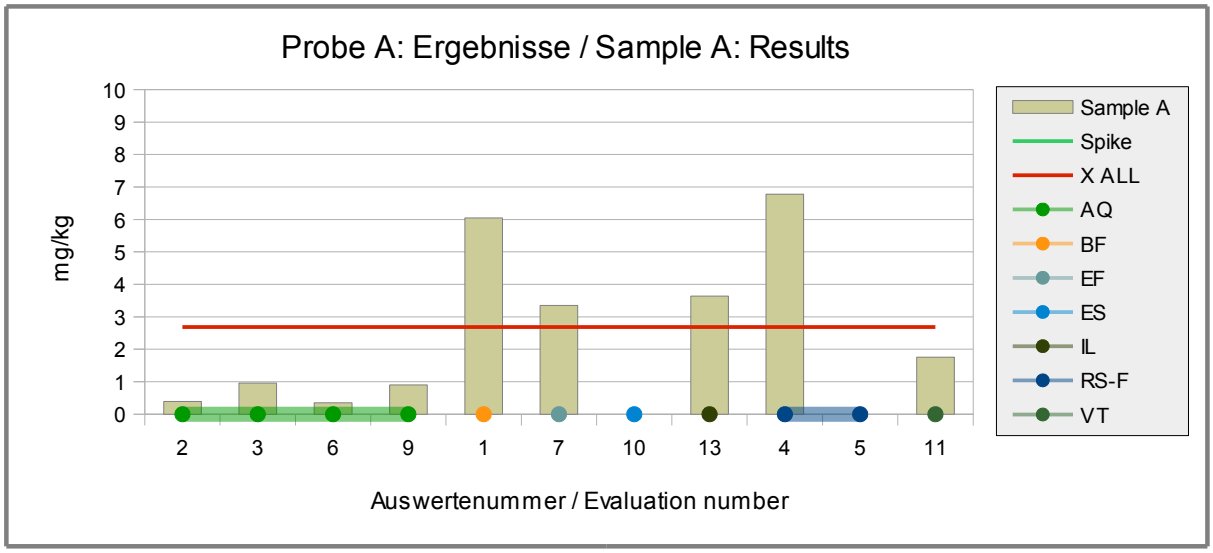


Abb./Fig. 2: ELISA Results Crustaceae (as total protein)
Spiking level (spike 59.1 mg / kg, not shown)
red line = Robust mean of all results (for information)
round symbols = Applied methods (see legend)

Quantitative valuation of results: Spiking level sample

Evaluation number	Crustaceae protein	Crustaceae protein	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
2	positive	0,560		AQ	
3	positive	1,32		AQ	Result converted °
6	positive	0,787		AQ	
9	positive	7,30		AQ	
1	positive	9,95		BF	Result converted °
7	positive	3,95		EF	Result converted °
10	positive	0,515		ES	Result converted °
13	positive	5,08		IL	Result converted °
4	positive	16,7		RS-F	Result converted °
5	positive	> 160		RS-F	Type of quantitative result unclear
11	positive	3,46		VT	Result converted °

° calculation see p. 19

Number positive	11	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methoden:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

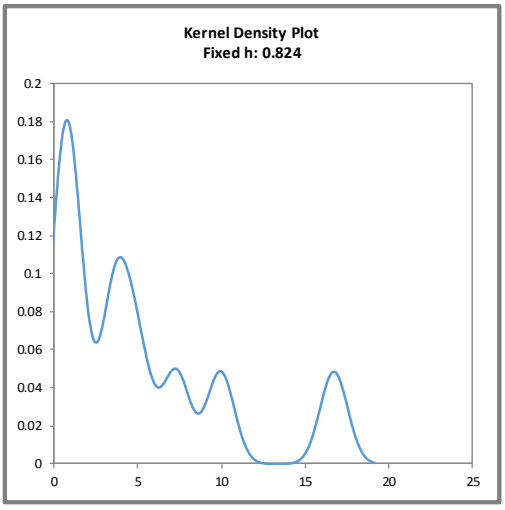


Abb. / Fig. 3:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation showed an inconsistent distribution of the results.

Characteristics: Quantitative evaluation Crustaceae (as total protein)

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	10
Number of outliers	
Mean	4,97
Median	3,71
Robust Mean (X)	**4,40**
Robust standard deviation (S*)	**4,43**
Target range:	
Target standard deviation σ_{pt}	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(X_{pt})}$	
Results in the target range	
Percent in the target range	

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a variety of maxima. A joint evaluation of the results of different methods was not possible. Due to the small number of results, no evaluation was made for single methods.
The individual quantitative results were evaluated in comparison to the spiking level for information (see "Recovery rates for Crustaceae (as total protein)" p.28).

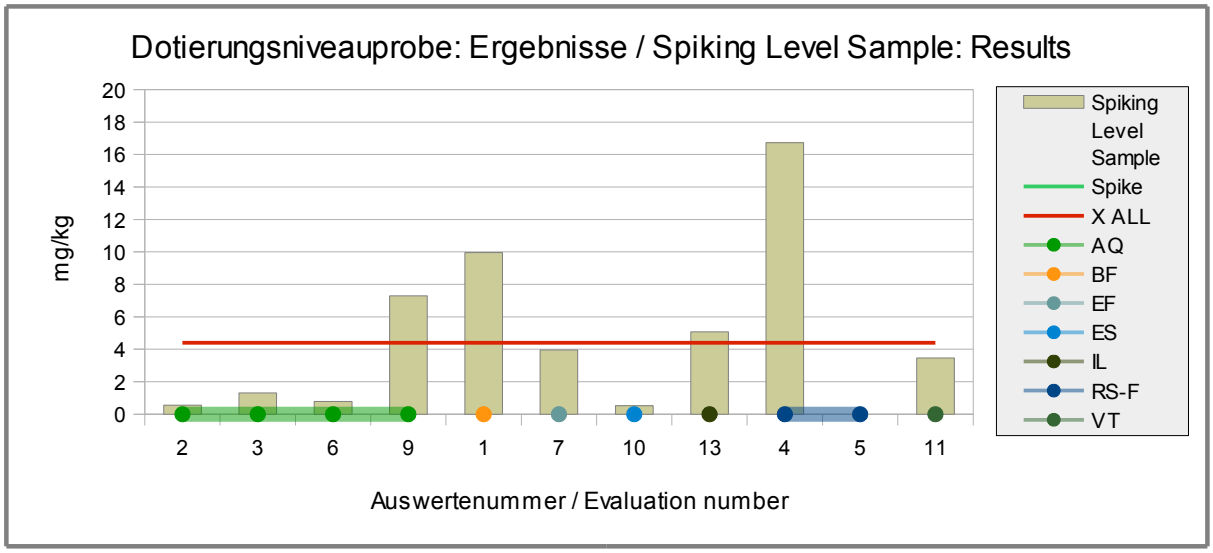


Abb./Fig. 4: ELISA Results Crustaceae (as total protein)
Spiking level (spike 57.1 mg / kg, not shown)
red line = Robust mean of all results (for information)
round symbols = Applied methods (see legend)

Recovery Rates ELISA for Crustaceae (as total protein): Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	0,560	1	0,390	1	AQ	
3	1,32	2	0,956	2	AQ	Result converted °
6	0,787	1	0,351	1	AQ	
9	7,30	13	0,900	2	AQ	
1	9,95	17	6,04	10	BF	Result converted °
7	3,95	7	3,35	6	EF	Result converted °
10	0,515	1	<0,25		ES	Result converted °
13	5,08	9	3,64	6	IL	Result converted °
4	16,7	29	6,78	11	RS-F	Result converted °
5	> 160				RS-F	
11	3,46	6	1,75	3	VT	Result converted °

° calculation see p. 19

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: Crustaceae protein, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

None of the participants has obtained a recovery rate within the range of the AOAC-recommendation of 50-150% with the spiking level sample or the spiked food matrix sample A by ELISA methods. The recovery rates ranged between 1-29% and 1-11% of the spiking level, respectively.

4.1.2 PCR Results: Crustaceae (dried)

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		
12	positive		negative		2/2 (100%)	ASU	
4	positive	12,4	negative	<0,22	2/2 (100%)	SFA	Result converted °
8	positive		negative		2/2 (100%)	SFA	

° calculation see p. 19

	Sample A		Sample B	
Number positive	3		0	
Number negative	0		3	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:
ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

Quantitative Valuation PCR: Sample A

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

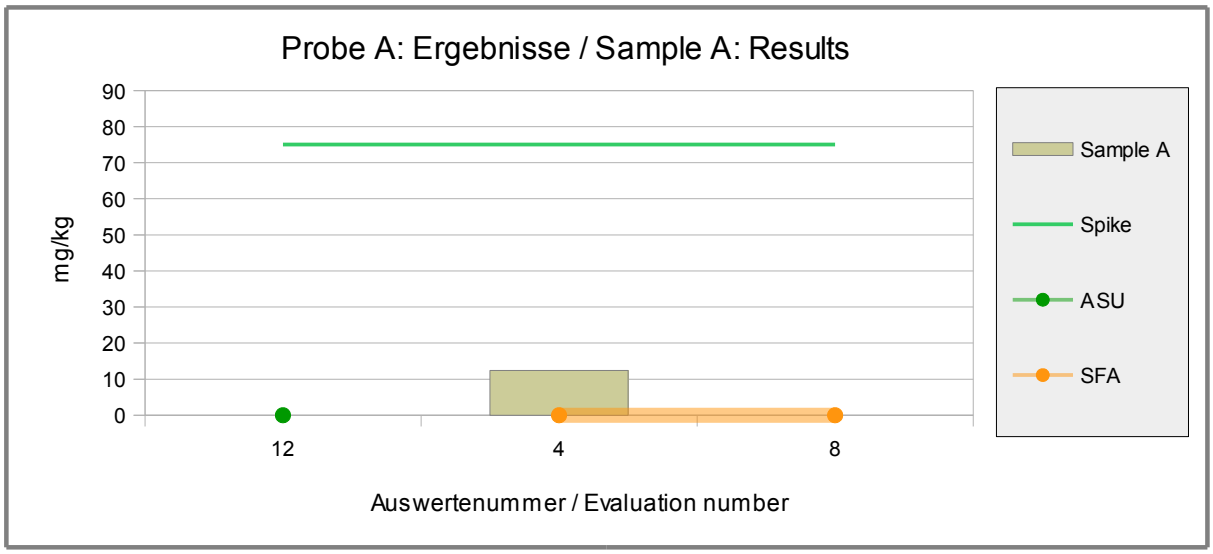


Abb./Fig. 5: PCR Results Crustaceae (dried)
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation PCR: Spiking Level Sample

No quantitative evaluation was done, because there were to few quantitative results.

Evaluation number	Crustaceae Protein	Crustaceae Protein	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
12	positive			ASU	
4	positive	13,1		SFA	Result converted °
8	positive			SFA	

° calculation see p. 19

Number positive	3	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methoden:
ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

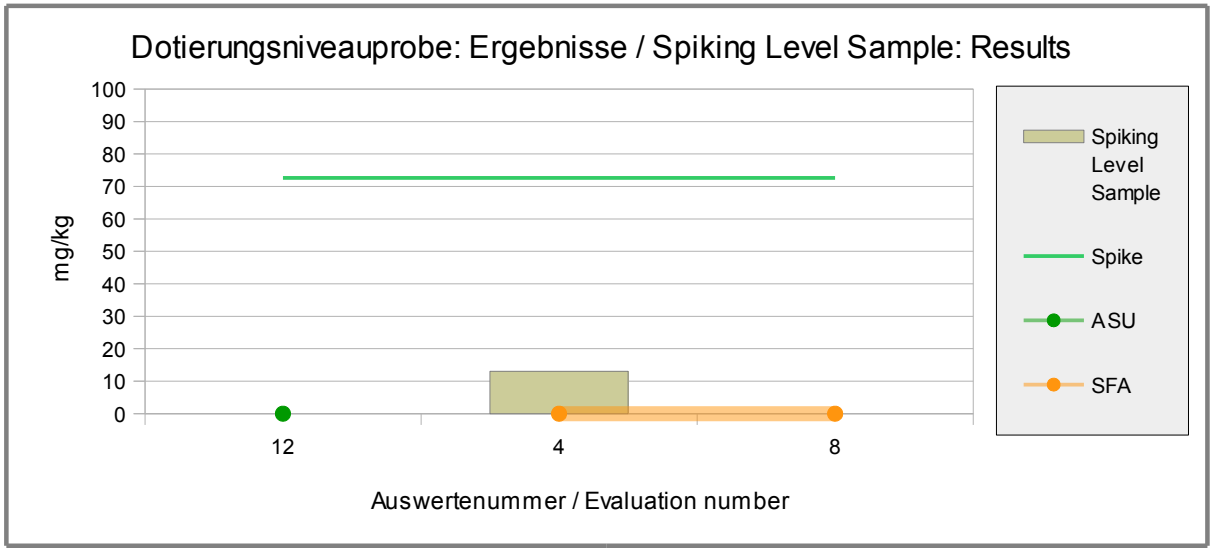


Abb./Fig. 6: PCR Results Crustaceae (dried)
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Crustaceae:
Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
12					ASU	
4	13,06	18	12,4	17	SFA	Result converted °
8					SFA	

° calculation see p. 19

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

Methods:
ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

** Range of acceptance of AOAC for allergen ELISAs

Comments:
One participant submitted quantitative results by PCR. The recovery rates were with 18% for the spiking material sample and 17% for the spiked food matrix sample A below the range of the AOAC-recommendation of 50-150%.

4.2 Proficiency Test Coconut

4.2.1 ELISA Results: Coconut, dried (Coconut flour)

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	646	negative	0	2/2 (100%)	BF	
7	positive	994	negative	<1,1	2/2 (100%)	EF	Result converted °
13	positive	519	negative	0	2/2 (100%)	IL	Result converted °

° calculation see p. 19

	Sample A		Sample B	
Number positive	3		0	
Number negative	0		3	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab

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

Quantitative valuation of ELISA-results: Sample A

No quantitative evaluation was done, because there were to few quantitative results.

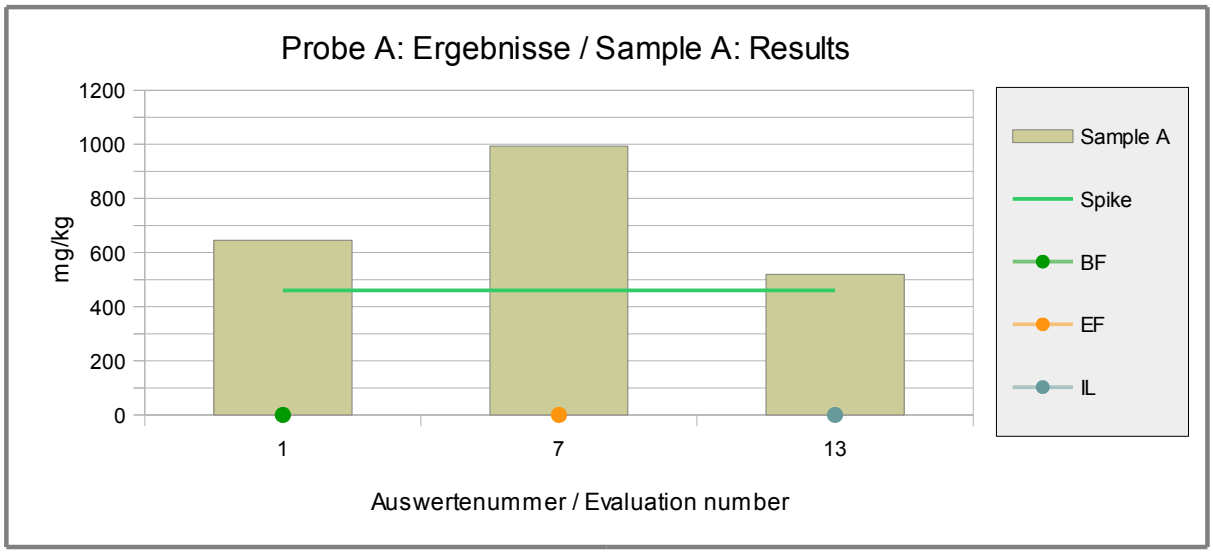


Abb./Fig. 7: ELISA Results Coconut (as coconut flour)
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation of results: Spiking level sample

No quantitative evaluation was done, because there were to few quantitative results.

Evaluation number	Coconut flour	Coconut flour	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positive	>1000		BF	
7	positive	828		EF	Result converted °
13	positive	487		IL	Result converted °

° calculation see p. 19

Number positive	3	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methoden:

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

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

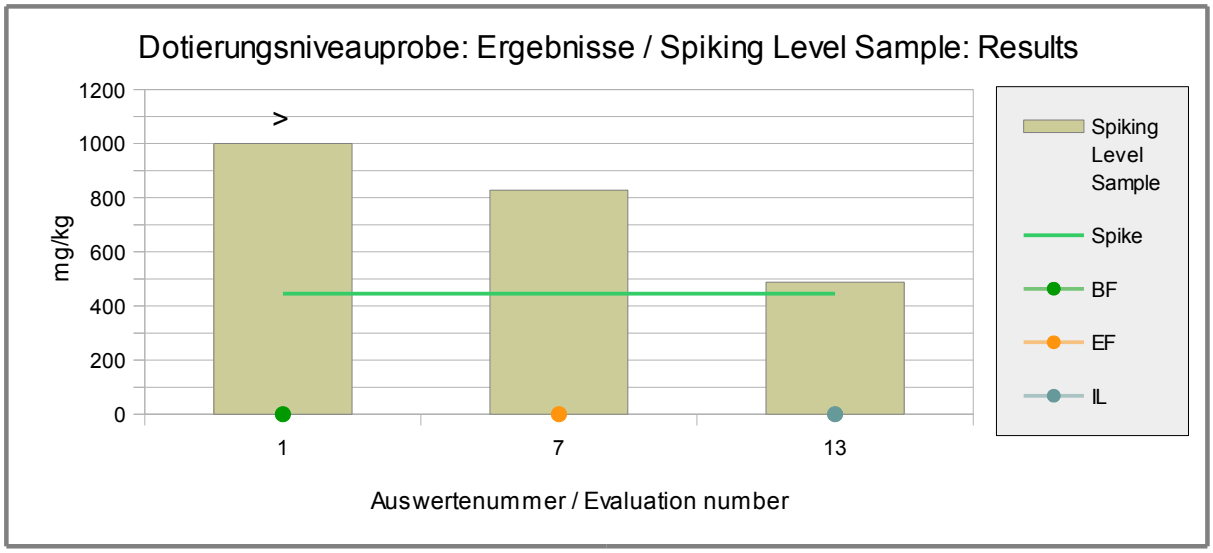


Abb./Fig. 8: ELISA Results Coconut (as coconut flour)
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates ELISA for Coconut:
Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	>1000		646	**140**	BF	
7	828	186	994	216	EF	Result converted °
13	487	**110**	519	**113**	IL	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**1**	Number in RA	**2**
Percent in RA	**50**	Percent in RA	**67**

Methods:
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
For the spiking level sample one of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A two of the recovery rates were within the range of acceptance.

4.2.2 PCR Results: Coconut

For coconut no PCR results were submitted.

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: Crustaceae

Meth. Abr.	Evaluation number	Date of analyses	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
AQ	2	19.01.00	positive	0,39	negative	<0.02	positive	0,56		0,02		Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AQ	3	01.12.18	Detected	1,21	Not Detected	<0.1	Detected	1,67	0,1	0,1	50	Crustacea	Romer
AQ	6	12.04.18	positive	0,351	negative	<0.02	positive	0,787		0,02		Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
AQ	9	28.12.18	positive	0,9	negative	<0.1	positive	7,3	0,0045	0,1		Crustacea protein	AgraQuant ELISA Crustacea COKAL2248, RomerLabs
BF	1	03.01.19	positive	35,1	negative	0	positive	57,8	0,07	1		Crustaceae, fresh	MonoTrace Crustacea ELISA kit, BioFront Technologies
EF	7	17.12.18	positive	0,67	negative	<0,02	positive	0,79	0,01	0,02		Tropomyosin Crustaceae	Eurofins SensiSpec Crustaceans (Tropomyosin) ELISA Kit
ES	10	17.12.18	negative	<0,05	negative	<0,05	positive	0,103	0,01	0,05	50	Tropomyosin protein	ELISA Systems Crustacean ESCRURD-48
IL	13	27.11.18	positive	0,727	negative	0	positive	1,015	0.0009	0,02		Tropomyosin	Immunolab Crustaceans (Tropomyosin) ELISA
RS-F	4	27.12.18	positive	33,9	negative	<20	positive	83,65	20	20	32,91	Crustaceae, fresh	Ridascreen® FAST Crustacean R7312, R-Biopharm
RS-F	5		negative		negative		positive	> 160	20	20	0,1	Please select!	Ridascreen® FAST Crustacean R7312, R-Biopharm
VT	11	11.12.18	positive	7,7	negative	<2.5	positive	15,2		2,5		Crustaceae, fresh	Veratox Crustacea, Neogen

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

Continuation ELISA Crustaceae:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	2				
AQ	3	Unknown	Tropomysin detection, reported as crustacea (not protein)	yes / no	
AQ	6			yes	
AQ	9				
BF	1	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 42C	no	
EF	7	detects crustaceaen tropomyosin	According to manufacturer's instructions	yes	
ES	10		According to kit-instruction.	yes	
IL	13	Tropomyosin			
RS-F	4	As per kit instructions	As per kit instructions	Yes	Variability between results was high for these samples
RS-F	5			yes	
VT	11			no	

5.1.2 ELISA: Coconut

Meth. Abr.	Evaluation number	Date of analyses	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
BF	1	03.01.19	positive	646	negative	0	positive	>1000	0,13	1		Coconut flour	MonoTrace Coconut ELISA kit, BioFront Technologies
EF	7	13.12.18	positive	1800	negative	<2	positive	1500	1,5	2		Coconut	Eurofins SensiSpec Coconut ELISA Kit
IL	13	27.11.18	positive	941	negative	0	positive	883	0.4	1		Coconut, fresh	Immunolab Coconut ELISA

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BF	1	monoclonal antibody-based kit	1:10 extraction ratio, 10 minutes at 60C	no	
EF	7	detects coconut protein	According to manufacturer's instructions	yes	
IL	13	total Coconut			

5.1.3 PCR: Crustaceae

Meth. Abr.	Evaluation number	Date of analyses	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
ASU	12		positive		negative		positive					Crustaceae-DNA	ASU §64 Methode/method
SFA	4	27.12.18	positive	56,86	negative	<1	positive	59,92	1	1	30	Crustaceae, fresh	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		positive		negative		positive		2			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ASU	12	16SrRNA	Extraction with Promega Wizard DNAcleanup100, 45 cycles, conventional PCR with sequence analysis	yes	Procambus clarkii
SFA	4	As per kit instructions	As per kit instructions	No	Variability between results was high for these samples
SFA	8		SUREFOOD PREP ADVANCED/TAQ POLIMERASA/STEP ONE APPLIED /40 CYCLES	no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 07-2018 Sample A

Weight whole sample	3,09	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	19,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	56	22,1
2	5,07	58	22,9
3	5,02	49	19,5
4	4,98	50	20,1
5	5,00	45	18,0
6	5,06	64	25,3
7	5,00	55	22,0
8	4,97	59	23,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,5	Particles
Standard deviation	5,99	Particles
χ^2 (CHI-Quadrat)	4,61	
Probability	**71**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	21,7	mg/kg
Standard deviation	2,39	mg/kg
rel. Standard deviaton	11,0	%
Horwitz standard deviation	10,1	%
HorRat-value	**1,1**	
Recovery rate	110	%

Microtracer Homogeneity Test
DLA 07-2018 Spiking Level Sample

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	37,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	111	44,4
2	5,01	114	45,5
3	5,02	112	44,6
4	5,05	101	40,0
5	5,00	114	45,6
6	5,02	107	42,6
7	4,99	105	42,1
8	5,02	103	41,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	108,4	Particles
Standard deviation	5,28	Particles
χ^2 (CHI-Quadrat)	1,80	
Probability	**97**	%
Recovery rate	115	%

Normal distribution		
Number of samples	8	
Mean	43,2	mg/kg
Standard deviation	2,11	mg/kg
rel. Standard deviaton	4,9	%
Horwitz standard deviation	9,1	%
HorRat-value	**0,5**	
Recovery rate	115	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 07-2018***
PT name	***Allergens VII: Crustaceae and Coconut in Instant Product with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *Tomato Soup Powder / ingredients: Tomatoes, starch, sugar, salt, rice flour, palm oil, onions, yeast extract, vegetable oil, spices, acidifier: citric acid, herbs, basil extract, other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* ***Crustaceae*** *(Crustaceae protein, DNA) added as* ***cooked, dried Crayfish****, and* ***Coconut*** *(Coconut protein, DNA) added as* ***Coconut flour*** *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.* *From* ***Samples A + B*** *the* ***total sample amount*** *should be* ***homogenized*** *each.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 04th January 2019***
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
		USA
		CANADA
		Germany
		SWEDEN
		GREAT BRITAIN
		CANADA
		Germany
		GREAT BRITAIN
		ITALY
		SPAIN
		Germany
		GREAT BRITAIN
		GREAT BRITAIN
		GREAT BRITAIN

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

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

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