Proficiency Tests
DLA
food
cosmetics
consumer goods
www.dla-lvu.de

Evaluation Report
proficiency test

DLA 04/2018

Allergens IV:

Celery, Mustard and Sesame

in Potato Chips

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

proficiency-testing@dla-lvu.de
www.dla-lvu.de

Coordinator of this PT:
Matthias Besler-Scharf, PhD

1st Correction 06/11/2018:
An error occurred in the tables of the PCR results for celery:
On page 22, for samples A and B the indication of the consensus values as negative and positive were mixed up in the last line of the table by mistake.
On page 24, the consensus value of the spiking level sample was indicated as negative by mistake. Both errors have been corrected.
The evaluations of the participants' results were not affected.

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

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler-Scharf Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA 04/2018
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (6 November 2018) 1. Korrektur / 1st Correction 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* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 6 November 2018
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.

Inhalt / Content

1. Introduction

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

2. Realisation

2.1 Test material

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

The test material were common in commerce potato chips. The basic composition of both sample A and sample B was the same (see table 1).
After crushing by a knife mill and sieving (mesh 2,5 mm) the basic mixture was homogenized.
Afterwards the **spiked sample B** was produced as follows:
The spiking materials (premix) containing the allergenic ingredients celery, mustard and sesame were crushed and sieved by means of a centrifugal mill (mesh 250 µm), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 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 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 level sample to approximately 15 g in metallized PET film bags.

Table 1: Composition of the DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Potato Chips light Ingredients: Potatoes, sunflower oil, salt Nutrients per 100g: Fat 22 g, carbohydrates 64 g, fiber 4,5 g, protein 7,0 g, salt 1,4 g	100 g/100 g	98,0 g/100g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	1,8 g/100 g	99,8 g/100 g
Celery seed: - as Celery seed powder* - *thereof 20,0% total protein***	-	 47,5 mg/kg 9,50 mg/kg	 46,3 mg/kg 9,27 mg/kg
Mustard, yellow (Sinapis alba): - as Mustard seed powder* - *thereof 30,6% total protein***	-	 60,3 mg/kg 18,5 mg/kg	 58,8 mg/kg 18,0 mg/kg
Sesame, white: - as Sesame seed* - *thereof 23,3% total protein***	-	 37,8 mg/kg 8,80 mg/kg	 36,8 mg/kg 8,58 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	<0,2 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 materials (total nitrogen according to Kjeldahl with F=6,25)

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].
Since the potato chips samples can not be assayed by microtracer analysis due to their fatty consistency, only the spiking level sample was measured. The microtracer analysis of the present PT sample showed a probability of 43%. 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 1,0. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample B by all ELISA tests for mustard (Immunolab, Veratox, AgraQuant) and sesame (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].

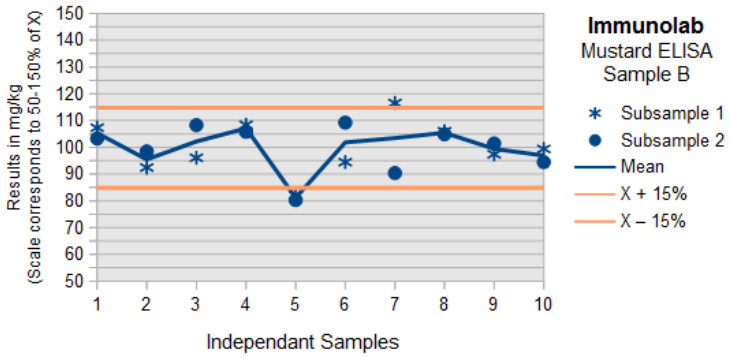
ELISA-Tests: Homogenität Senf / Homogeneity Mustard

Immunolab Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 99,8 ± 6,5 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	107	103	105
2	92,5	98,5	95,5
3	96,1	108	102
4	108	106	107
5	82,1	80,3	81,2
6	94,5	109	102
7	117	90,4	103
8	106	105	105
9	97,4	101	99,4
10	99,4	94,5	96,9

General average X	99,8	
SD of sample means Sx	7,54	7,6%
SD within-samples Sw	5,37	5,4%
SD between-samples Ss	6,51	6,5%

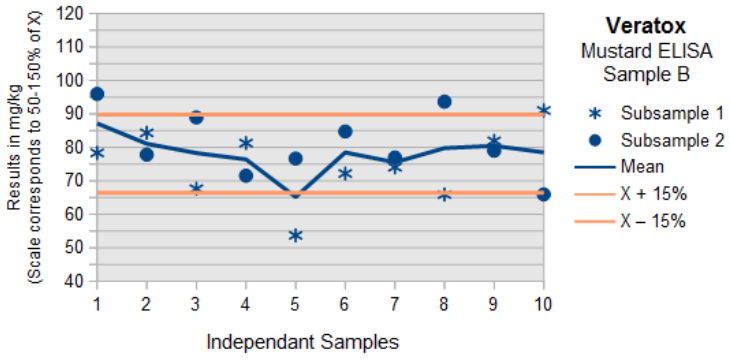


Neogen Veratox ELISA Mustard

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Mustard 78,1 ± 5,5 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	78,3	95,9	87,1
2	84,3	77,8	81,1
3	67,6	88,9	78,3
4	81,2	71,5	76,4
5	53,7	76,6	65,2
6	72,2	84,7	78,5
7	74,0	77,0	75,5
8	65,9	93,6	79,8
9	81,9	79,0	80,5
10	91,0	65,9	78,5

General average X	78,1	
SD of sample means Sx	5,54	7,1%
SD within-samples Sw	8,66	11,1%
SD between-samples Ss	< 5,54	< 7,1%

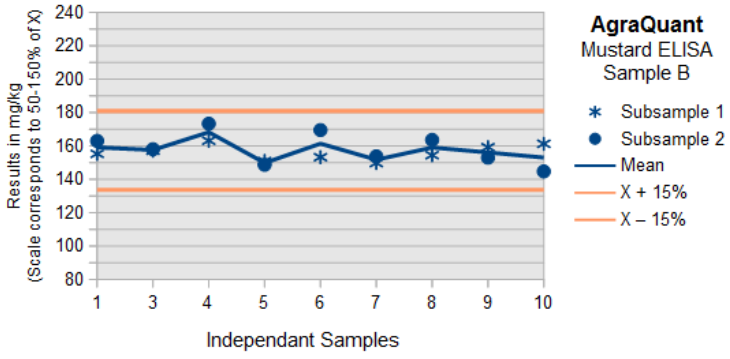


Romerlabs AgraQuant Mustard

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 157 ± 4,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	155	163	159
3	157	158	158
4	163	173	168
5	151	149	150
6	153	169	161
7	150	154	152
8	154	164	159
9	159	153	156
10	161	145	153

General average X	157	
SD of sample means Sx	5,58	3,5%
SD within-samples Sw	4,85	3,1%
SD between-samples Ss	4,40	2,8%



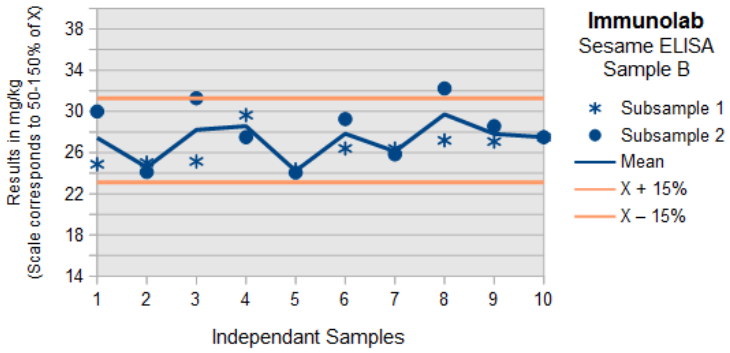
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Immunolab Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 27,2 ± 1,3 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	24,9	30,0	27,4
2	25,0	24,1	24,6
3	25,1	31,3	28,2
4	29,6	27,5	28,6
5	24,4	24,1	24,2
6	26,4	29,3	27,8
7	26,4	25,8	26,1
8	27,2	32,2	29,7
9	27,1	28,6	27,8
10	27,5	27,5	27,5

General average X	27,2	
SD of sample means Sx	1,73	6,4%
SD within-samples Sw	1,62	6,0%
SD between-samples Ss	1,30	4,8%

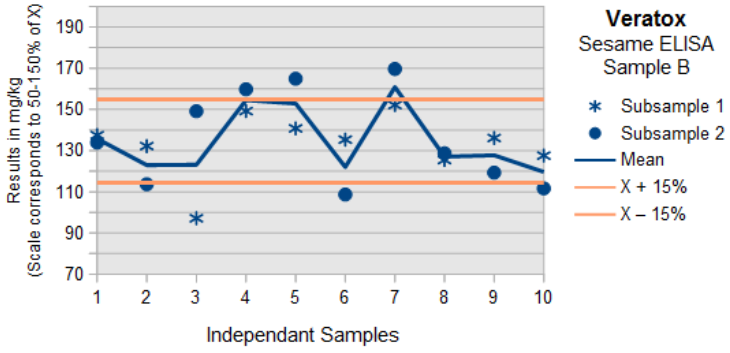


Neogen Veratox ELISA Sesame

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Sesame 136 ± 13,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	138	134	136
2	132	114	123
3	97	149	123
4	149	160	154
5	141	165	153
6	135	109	122
7	152	170	161
8	125	129	127
9	136	119	128
10	128	112	120

General average X	135	
SD of sample means Sx	15,5	11,5%
SD within-samples Sw	11,5	8,6%
SD between-samples Ss	13,2	9,8%

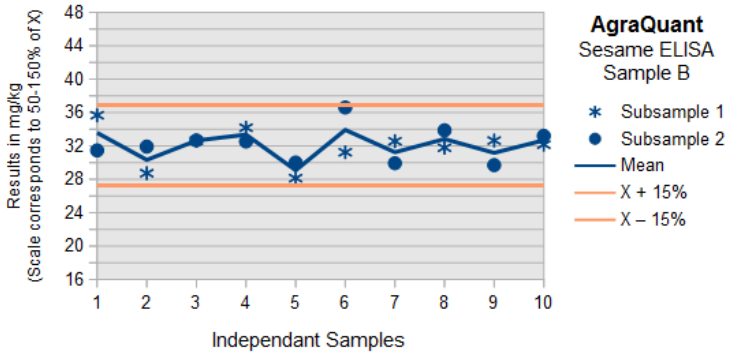


Romerlabs AgraQuant Sesame

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

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	35,7	31,5	33,6
2	28,7	31,9	30,3
3	32,6	32,7	32,6
4	34,2	32,5	33,3
5	28,1	30,0	29,1
6	31,2	36,6	33,9
7	32,6	29,9	31,2
8	31,8	33,9	32,8
9	32,6	29,7	31,2
10	32,1	33,2	32,7

General average X	32,1	
SD of sample means Sx	1,57	4,9%
SD within-samples Sw	1,45	4,5%
SD between-samples Ss	1,19	3,7%



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. 0,17 (25,8°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 24th week of 2018. The testing method was optional. The tests should be finished at August 10th 2018.

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 Celery, Mustard and/or Sesame in the range of mg/kg in the matrix of Potato Chips. 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 sent 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, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 40 participants submitted their results in time.

3. Evaluation

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

For quantitative results of the spiking 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, and results for a 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	σ_{pt}	Method / Literature
Peanut	Milk chocolate	173,7 33,8 5,9	87 % 85 % 59 %	- - -	8,8% 5,2% 7,8%	31% 20% 31%	30,4% 19,7% 30,5%	ELISA Manuf. A ASU 00.00-69
Peanut	Milk chocolate	215,7 40,1 10,1	108 % 100 % 101 %	- - -	5,9% 7,2% 7,3%	32% 14% 16%	31,7% 13,0% 15,1%	ELISA Manuf. B ASU 00.00-69
Peanut	Dark chocolate	148,2 30,9 5,7	74 % 77 % 57 %	- - -	6,0% 13% 6,1%	22% 25% 33%	21,6% 23,2% 32,7%	ELISA Manuf. A ASU 00.00-69
Hazelnut	Dark chocolate	16,3 7,56 3,73 1,62	81 % 76 % 75 % 81 %	- - - -	4,7% 8,9% 13% 15%	12% 15% 24% 33%	11,5% 13,6% 22,2% 31,2%	ELISA Manuf. A ASU 44.00-7
Hazelnut	Dark chocolate	21,3 10,7 4,69 2,37	106 % 107 % 94 % 119 %	- - - -	7,1% 11% 11% 9,3%	14% 19% 17% 17%	13,1% 17,3% 15,1% 16,4%	ELISA Manuf. B ASU 44.00-7

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 11 – 32% for the ELISA methods and 18 – 38% 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**
Celery seed	Sausage, cooked (100°C, 60 min)	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 seed	Sausage, autoclaved	10,5	10,5 %	-	25,8%	39,4%	34,9%	rt-PCR ASU 08.00-65
Mustard, brown / black	Sausage, autoclaved	146,7 50,0 15,8	147 % 125 % 158 %	-	12,3% 17,2% 15,4%	22,0% 31,6% 27,1%	20,2% 29,2% 24,8%	rt-PCR ASU 08.00-64
Mustard, brown / black	Sausage, autoclaved	168,3 52,9 17,6	168 % 132 % 176 %	-	11,4% 10,0% 23,1%	31,6% 23,1% 46,3%	29,5% 21,9% 43,3%	rt-PCR ASU 08.00-65
Mustard, white	Sausage, cooked (100°C, 60 min)	79,9 37,0 18,0 8,0	80 % 93 % 90 % 80 %	-	13,6% 15,7% 14,4% 15,4%	23,6% 29,2% 30,6% 26,1%	21,6% 27,0% 28,9% 23,7%	rt-PCR ASU 08.00-59
Mustard, white	Sausage, cooked (100°C, 60 min)	103,3 45,9	103 % 115 %	- -	11,8% 14,7%	17,1% 21,8%	14,9% 19,2%	rt-PCR ASU 08.00-65
Mustard, white	Sausage, autoclaved	11,7	11,7 %	-	24,1%	34,3%	29,8%	rt-PCR ASU 08.00-65
Sesame	Rice cookie	94,6 15,7 9,8	95 % 79 % 98 %	-	22,5% 26,0% 20,9%	27,5% 39,5% 33,5%	22,4% 35,0% 30,0%	rt-PCR ASU 18.00-19
Sesame	Wheat cookie Sauce powder	96,9 59,8	79 % 60 %	-	21,8% 22,2%	33,0% 43,2%	29,2% 40,2%	rt-PCR ASU 18.00-19
Sesame	Rice cookie	88,9 17,8 9,8	89 % 89 % 98 %	-	18,2% 34,2% 26,2%	30,5% 37,8% 37,0%	27,7% 29,1% 32,0%	rt-PCR ASU 18.00-22
Sesame	Wheat cookie Sauce powder	115 58,5	93 % 59 %	-	16,7% 30,8%	41,1% 44,4%	39,4% 38,7%	rt-PCR ASU 18.00-22

3.4.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].
Criteria for the level of performance of analytical methods for the quantitative determination of allergens in foods were recently elaborated e.g. by the Ministry of Health and Welfare (MHLW) in Japan [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 process, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision, and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [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 (xi) 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 (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*/σ_{pt}

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

3.8 Standard uncertainty 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

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.

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

In the present PT, the quantitative PCR results e.g. for celery were sometimes given unclear or implausible as DNA, seed, tuber and/or only as celery, mustard and sesame. It was therefore not intended to standardize the PCR results.

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

° Target range is calculated with 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 Celery

4.1.1 ELISA Results: Celery (Celery seed)

Comments:
None of the participants used the ELISA method for determination of celery.

4.1.2 PCR Results: Celery (Celery seed)

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		positive	61,9	2/2 (100%)	ASU	as celery
24	negative		positive	38,7	2/2 (100%)	ASU	as celery seed, dried
28	negative		positive		2/2 (100%)	ASU	
31	negative		positive		2/2 (100%)	ASU	
34	negative		positive		2/2 (100%)	ASU	
37	negative		positive		2/2 (100%)	ASU	
4	negative		positive		2/2 (100%)	FP	as celery DNA
7	negative		positive		2/2 (100%)	GI	
32	negative		positive	170	2/2 (100%)	MS	as celery DNA
2	negative	< 1,0	positive	8,75	2/2 (100%)	SFA	as celery DNA
26	negative		positive		2/2 (100%)	SFA-4p	
8	negative	< 0.4	positive	> 0.4	2/2 (100%)	SFA-ID	as celery
12	negative	< 1	positive	119	2/2 (100%)	SFA-ID	as celery
22	negative		positive		2/2 (100%)	SFA-ID	
23	negative		positive		2/2 (100%)	SFA-ID	
14	negative	< 0,4	positive	1,60	2/2 (100%)	SFA-Q	as celery
5	negative		positive		2/2 (100%)	div	
6	negative		positive		2/2 (100%)	div	
17	-		positive		1/1 (100%)	div	
19	negative		positive		2/2 (100%)	div	
21	negative		positive	15,0	2/2 (100%)	div	as celery DNA, celery tuber
25	negative		positive		2/2 (100%)	div	
30	negative		positive		2/2 (100%)	div	
35	negative		positive		2/2 (100%)	div	
40	negative	< 50	positive	100	2/2 (100%)	div	as celery

	Sample A		Sample B	
Number positive	0		25	
Number negative	24		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GI = GEN-IAL First Allergen
MS = Microsynth
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
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done. Moreover the quantitative PCR results for celery were sometimes unclear or implausible given as celery DNA, celery seed, celery tuber and/or as celery only.

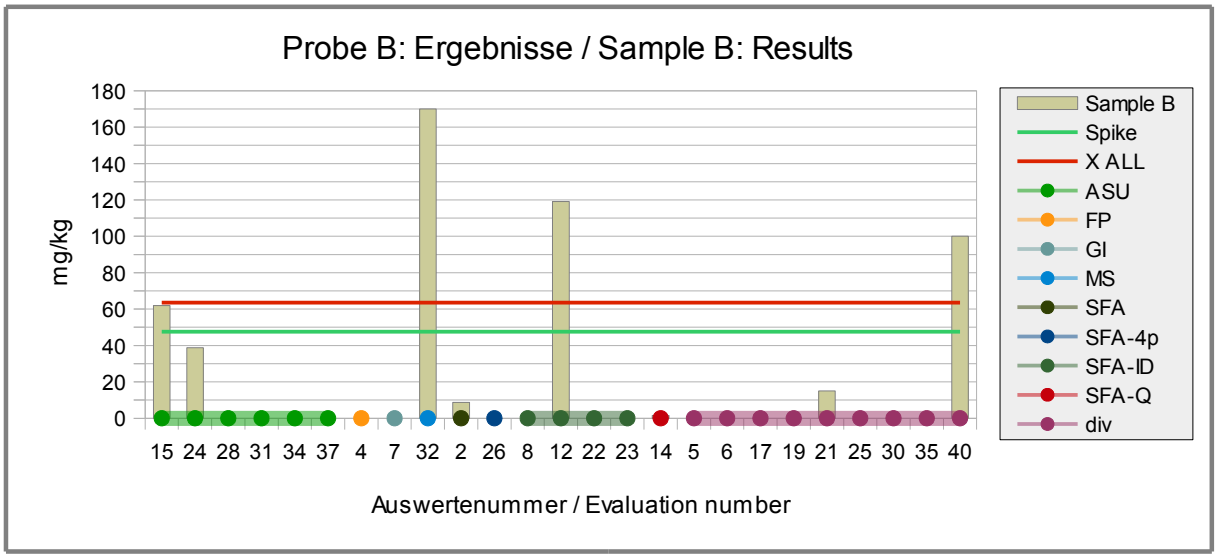


Abb./Fig. 1: PCR Results Celery (Sample B)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

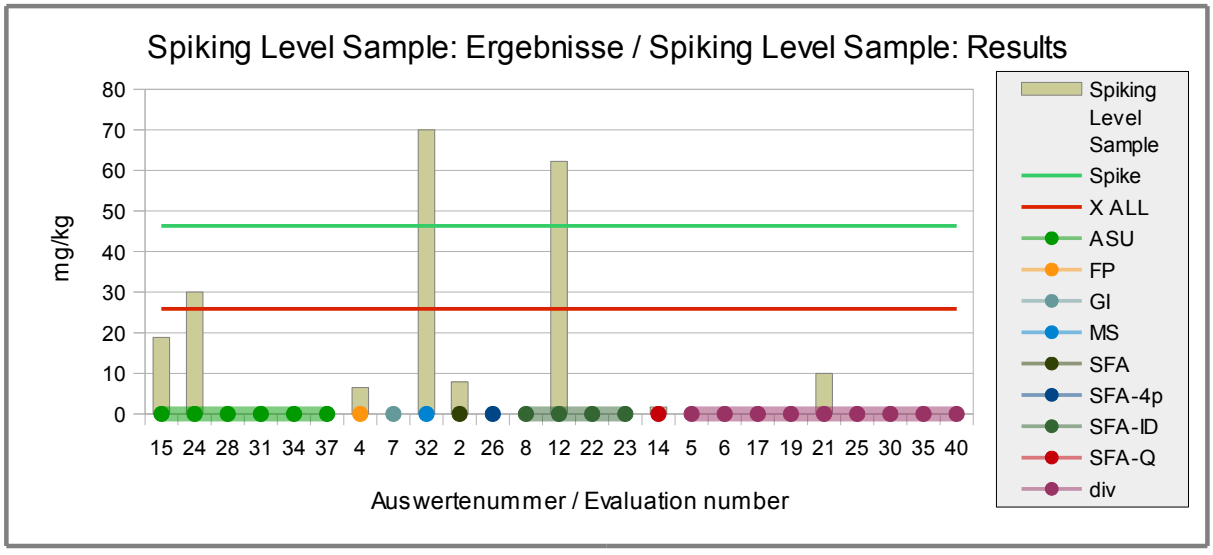


Abb./Fig. 2: PCR Results Celery (Spiking Level Sample)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done. Moreover the quantitative PCR results for celery were sometimes unclear or implausible given as celery DNA, celery seed, celery tuber and/or as celery only.

Evaluation number	Celery	Celery	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
15	positive	18,8		ASU	as celery
24	positive	30,0		ASU	as celery seed, dried
28	positive			ASU	
31	positive			ASU	
34	positive			ASU	
37	positive			ASU	
4	positive	6,47		FP	as celery DNA
7	negative			GI	
32	positive	70,0		MS	as celery DNA
2	positive	7,88		SFA	as celery DNA
26	positive			SFA-4p	
8	positive	> 0.4		SFA-ID	as celery
12	positive	62,2		SFA-ID	as celery
22				SFA-ID	
23	positive			SFA-ID	
14	positive	1,68		SFA-Q	as celery
5	positive			div	
6	positive			div	
17				div	
19	positive			div	
21	positive	10		div	as celery DNA, celery tuber
25	positive			div	
30	positive			div	
35	positive			div	
40	positive	<100		div	as celery

Number positive	22	
Number negative	1	
Percent positive	96	
Percent negative	4	
Consensus value	positive	

Methods:
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GI = GEN-IAL First Allergen
MS = Microsynth
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
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Recovery Rates PCR for Celery (informative only): Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
15	18,8	41	61,9	130	ASU	as celery
24	30,0	65	38,7	81	ASU	as celery seed, dried
28					ASU	
31					ASU	
34					ASU	
37					ASU	
4	6,47	14			FP	as celery DNA
7					GI	
32	70,0	151	170	358	MS	as celery DNA
2	7,88	17	8,75	18	SFA	as celery DNA
26					SFA-4p	
8	> 0.4		> 0.4		SFA-ID	as celery
12	62,2	134	119	251	SFA-ID	as celery
22					SFA-ID	
23					SFA-ID	
14	1,68	4	1,60	3	SFA-Q	as celery
5					div	
6					div	
17					div	
19					div	
21	10,0	22	15,0	32	div	as celery DNA, celery tuber
25					div	
30					div	
35					div	
40	<100		100	210	div	as celery

RA**	50-150 %	RA**	50-150 %
Number in RA	2	Number in RA	2
Percent in RA	25	Percent in RA	25

* 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
GI = GEN-IAL First Allergen
MS = Microsynth
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
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Comments:

The indication of the recovery rates for celery by PCR determination is exclusively informative, because on one hand the reference is celery seed (see p.5) and on the other hand partly other references are indicated for participants' results and partly the reference given is not plausible.

4.2 Proficiency Test Mustard

4.2.1 ELISA Results: Mustard (Sinapis alba)

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		
6	negative	< 2	positive	48,4	2/2 (100%)	AQ	
7	negative	0	positive	> 40	2/2 (100%)	AQ	
33	negative	< 2	positive	42,9	2/2 (100%)	AQ	
29	negative	< 2	positive	89,6	2/2 (100%)	BC	
13	negative	0	positive	93,5	2/2 (100%)	BF	
8	negative	< 1	positive	49,0	2/2 (100%)	ES	result converted °
38	negative	<1.2	positive	100	2/2 (100%)	IL	
2	negative	< 0,5	positive	72,8	2/2 (100%)	RS-F	
5	negative	< 0,5	positive	56,9		RS-F	
14	negative	< 0,1	positive	83,5	2/2 (100%)	RS-F	
17	negative		positive		2/2 (100%)	RS-F	
20	negative	< 0,5	positive	> 13,5	2/2 (100%)	RS-F	
23	negative	< 0,5	positive	83,0	2/2 (100%)	RS-F	
28	negative	< LOQ	positive	110		RS-F	
31	negative		positive	96,0	2/2 (100%)	RS-F	
9	negative	< 2,5	positive	50,1	2/2 (100%)	VT	
10	negative	< 2,5	positive	94,0	2/2 (100%)	VT	
11	negative	< 2,5	positive	109		VT	
12	negative	< 2.5	positive	82,0	2/2 (100%)	VT	
18	negative	< LOD	positive	267	2/2 (100%)	VT	result converted °
27	negative	< 2,5	positive	110	2/2 (100%)	VT	
30	negative	< 2,5	positive	66,0	2/2 (100%)	VT	
36	negative	ND	positive	38,0	2/2 (100%)	VT	
40	negative	< 2,5	positive	75,0	2/2 (100%)	VT	

° calculation p. 19

	Sample A		Sample B	
Number positive	0		24	
Number negative	24		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

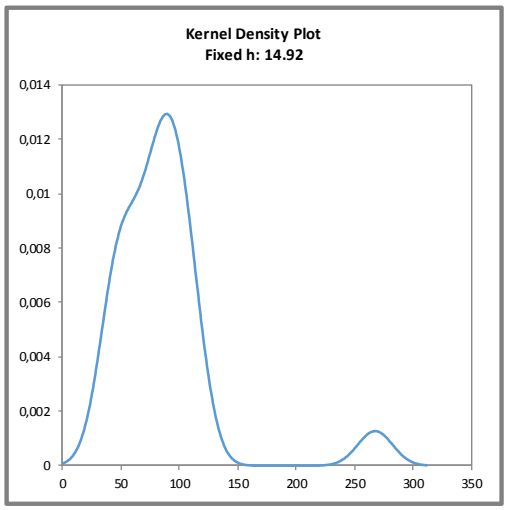
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of ELISA results: Sample B

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
6	48,4	-1,6			AQ	
7	> 40				AQ	
33	42,9	-1,8			AQ	
29	89,6	0,50			BC	
13	93,5	0,70			BF	
8	49,0	-1,5			ES	result converted °
38	100	1,0			IL	
2	72,8	-0,34	-0,52		RS-F	
5	56,9	-1,1	-1,3		RS-F	
14	83,5	0,20	-0,01		RS-F	
17					RS-F	
20	> 13,5				RS-F	
23	83,0	0,17	-0,03		RS-F	
28	110	1,5	1,3		RS-F	
31	96,0	0,83	0,59		RS-F	
9	50,1	-1,5		-1,6	VT	
10	94,0	0,72		0,44	VT	
11	109	1,5		1,1	VT	
12	82,0	0,12		-0,13	VT	
18	267	9,4		8,6	VT	result converted °
27	110	1,5		1,2	VT	
30	66,0	-0,68		-0,88	VT	
36	38,0	-2,1		-2,2	VT	
40	75,0	-0,23		-0,46	VT	

° calculation p. 19



Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
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 shows nearly a symmetrical distribution of results with a shoulder at approx. 50 mg/kg with single results of the

methods AQ, ES, RS-F and VT and a side peak at 270 mg/kg (single result of method VT, eventually submitted as protein by mistake) (see fig.3).

Characteristics: Quantitative evaluation ELISA Mustard

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*	**Method VT** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$	$X_{pt_{METHOD\ VT}}$
Number of results	21	6	9
Number of outliers	-	-	-
Mean	86,5	83,7	99,0
Median	83,0	83,3	82,0
Robust Mean (X_{pt})	**79,6**	**83,7**	**84,7**
Robust standard deviation (S*)	**28,1**	**20,8**	**36,0**
Target range:			
Target standard deviation σ_{pt}	**19,9**	**20,9**	**21,2**
lower limit of target range	**39,8**	**41,9**	**42,4**
upper limit of target range	**119**	**126**	**127**
Quotient S/σ_{pt}*	*1,4*	*0,99*	*1,7*
Standard uncertainty $U_{(X_{pt})}$	*7,68*	*10,6*	*15,0*
Results in the target range	*19*	*6*	*7*
Percent in the target range	*90*	*100*	*78*

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

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences.

The evaluations of the results of all methods and the results from methods RS-F and VT showed all a low to normal variability of results. The quotients S/σ_{pt} were each below 2,0.*
The robust standard deviation is in the range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 132%, 139% and 140% of the spiking level of mustard to sample B and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for mustard", p.35).

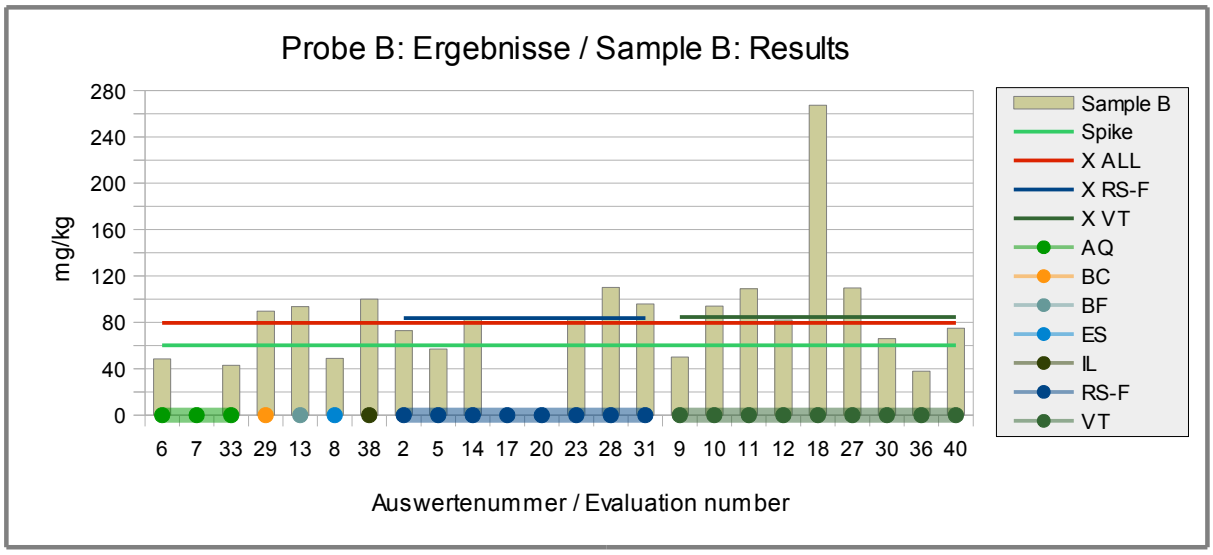


Abb./Fig. 4: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

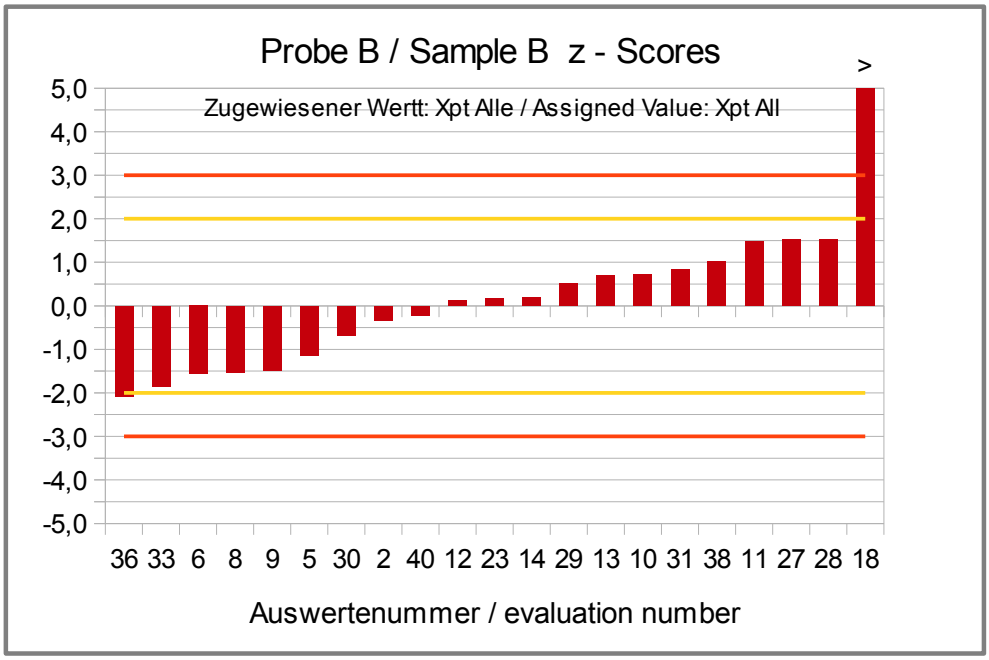


Abb./Fig. 5:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

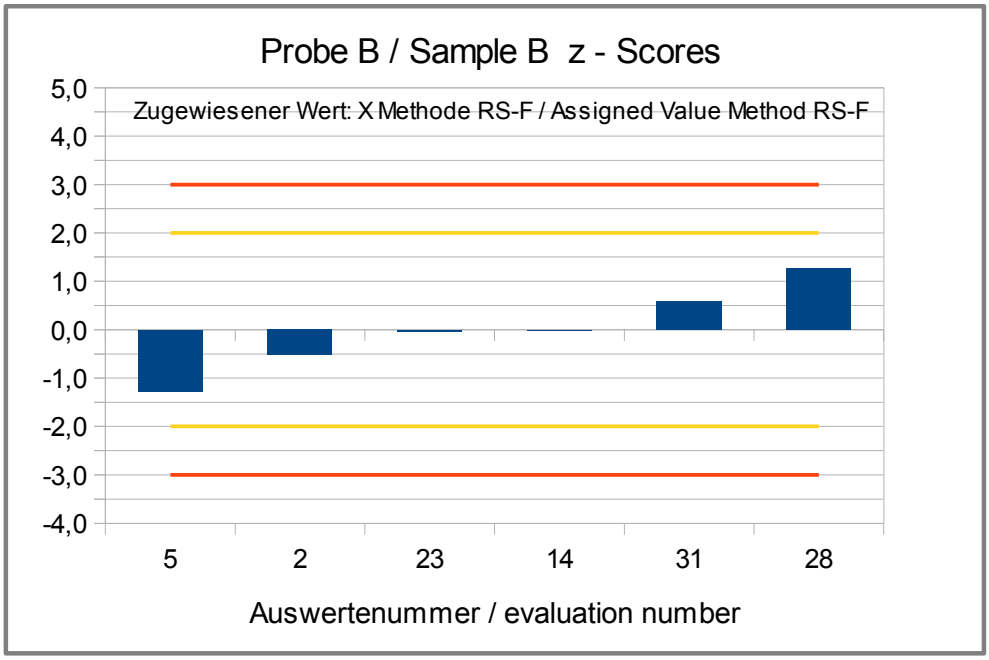


Abb./Fig. 6:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen)

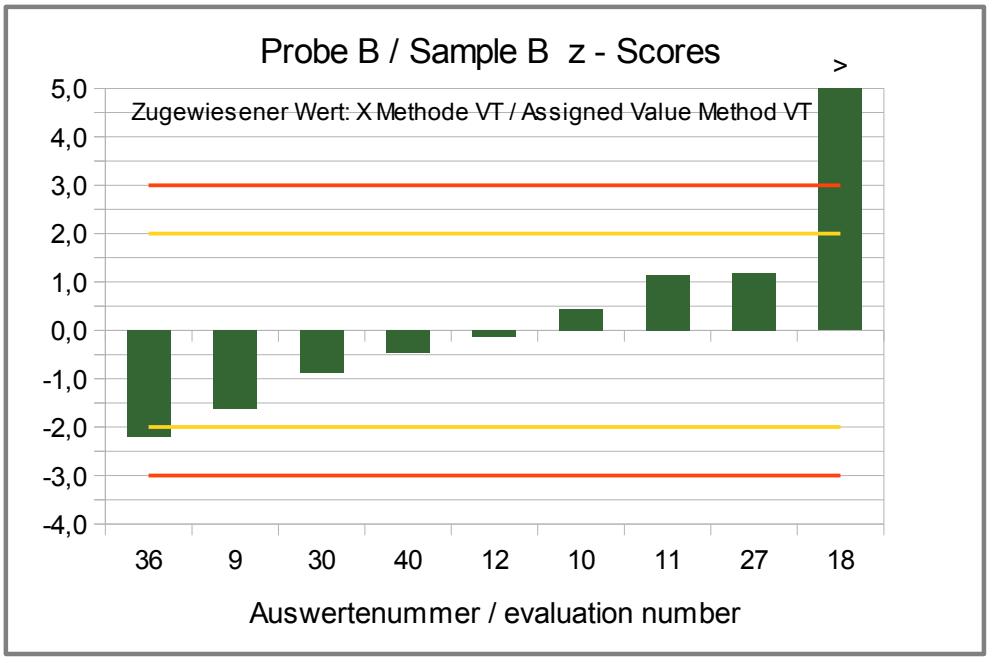
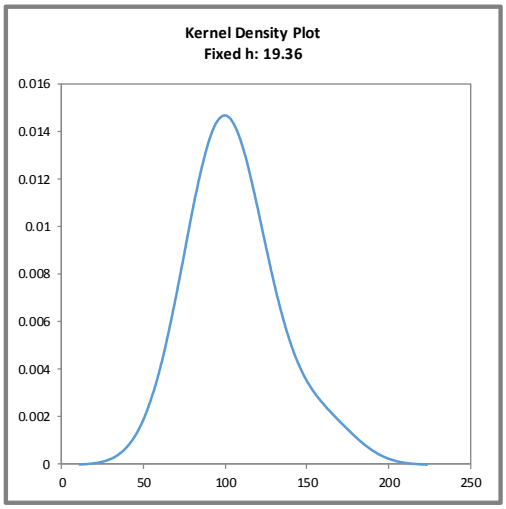


Abb./Fig. 7:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

Quantitative valuation of ELISA results: Spiking level sample

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
6	166	2,4			AQ	
7	> 40				AQ	
33	151	1,8			AQ	
29	101	-0,09			BC	
13	87,9	-0,59			BF	
8	68,6	-1,3			ES	result converted °
38	125	0,84			IL	
2	108	0,18	0,22		RS-F	
5	99,2	-0,16	-0,12		RS-F	
14	112	0,33	0,37		RS-F	
17					RS-F	
20	> 13,5				RS-F	
23	79,0	-0,94	-0,91		RS-F	
28	110	0,27	0,31		RS-F	
31	98,9	-0,17	-0,13		RS-F	
9	84,0	-0,75		-0,66	VT	
10	118	0,55		0,68	VT	
11	126	0,88		1,0	VT	
12	102	-0,05		0,06	VT	
18					VT	
27	93,7	-0,37		-0,27	VT	
30	100	-0,13		-0,02	VT	
36	87,0	-0,63		-0,54	VT	
40	95,0	-0,32		-0,22	VT	

° calculation p. 19



Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

Abb. / Fig. 8:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

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

Characteristics: Quantitative evaluation ELISA Mustard

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*	**Method VT** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$	$X_{pt_{METHOD\ VT}}$
Number of results	20	6	8
Number of outliers	–	–	–
Mean	106	101	101
Median	100	104	97,5
Robust Mean (X_{pt})	**103**	**102**	**100**
Robust standard deviation (S*)	**19,4**	**11,1**	**16,0**
Target range:			
Target standard deviation σ_{pt}	**25,8**	**25,6**	**25,1**
lower limit of target range	**51,6**	**51,2**	**50,2**
upper limit of target range	**155**	**153**	**151**
Quotient S/σ_{pt}*	*0,75*	*0,43*	*0,64*
Standard uncertainty $U_{(Xpt)}$	*5,41*	*5,65*	*7,08*
Results in the target range	*19*	*6*	*8*
Percent in the target range	*95*	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences.

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

The robust means of the evaluations were 175%, 173% and 170% of the spiking level of mustard to the spiking level sample and thus above the recommendations for the applied methods (s. 3.4.3 and "recovery rates for mustard", p.35).

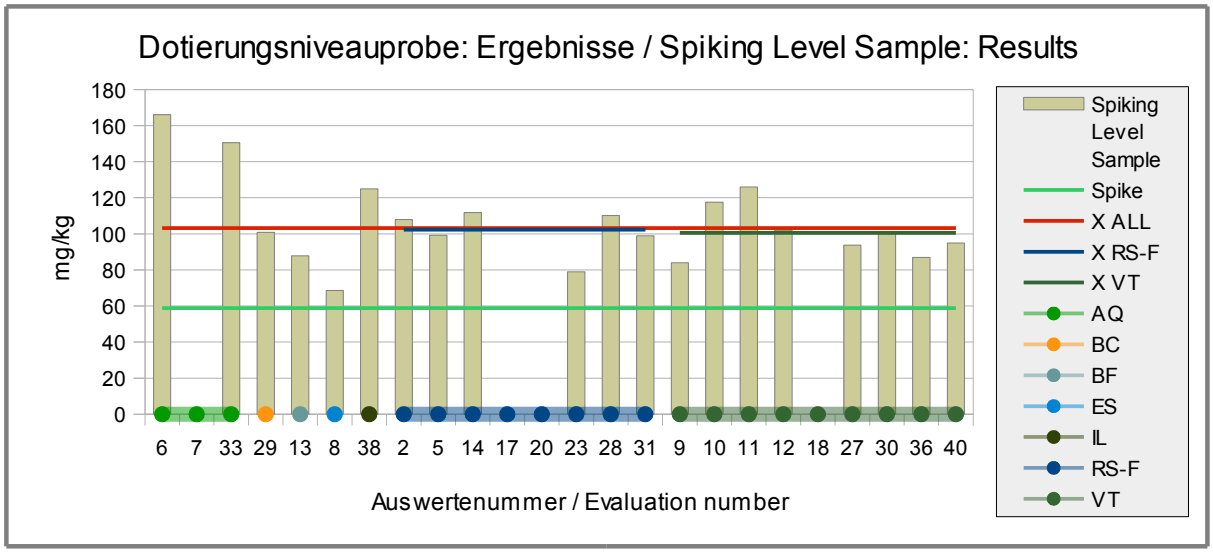


Abb./Fig. 9: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

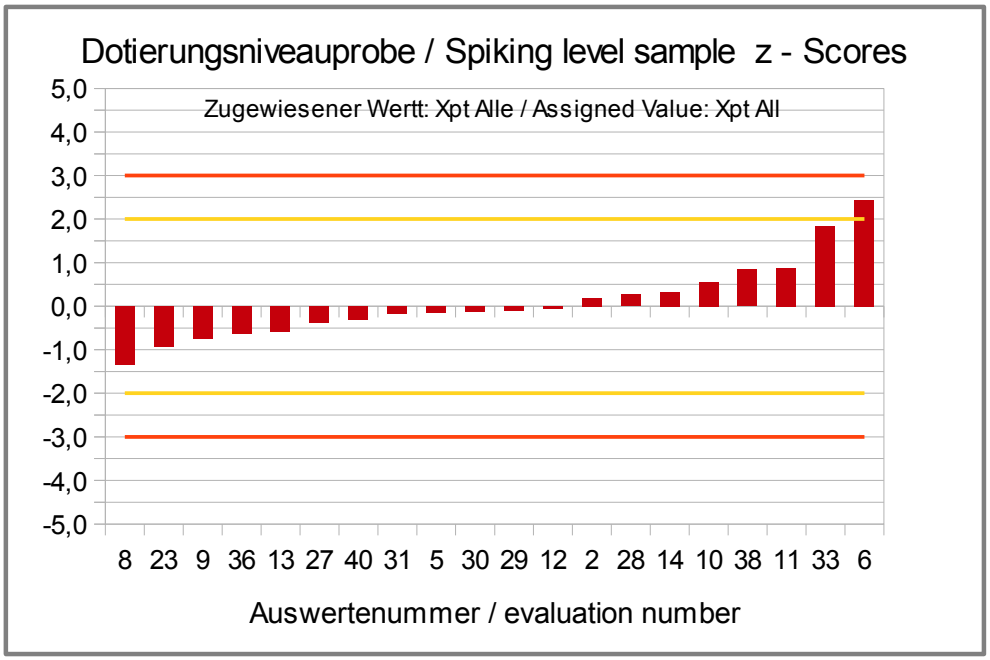


Abb./Fig. 10:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

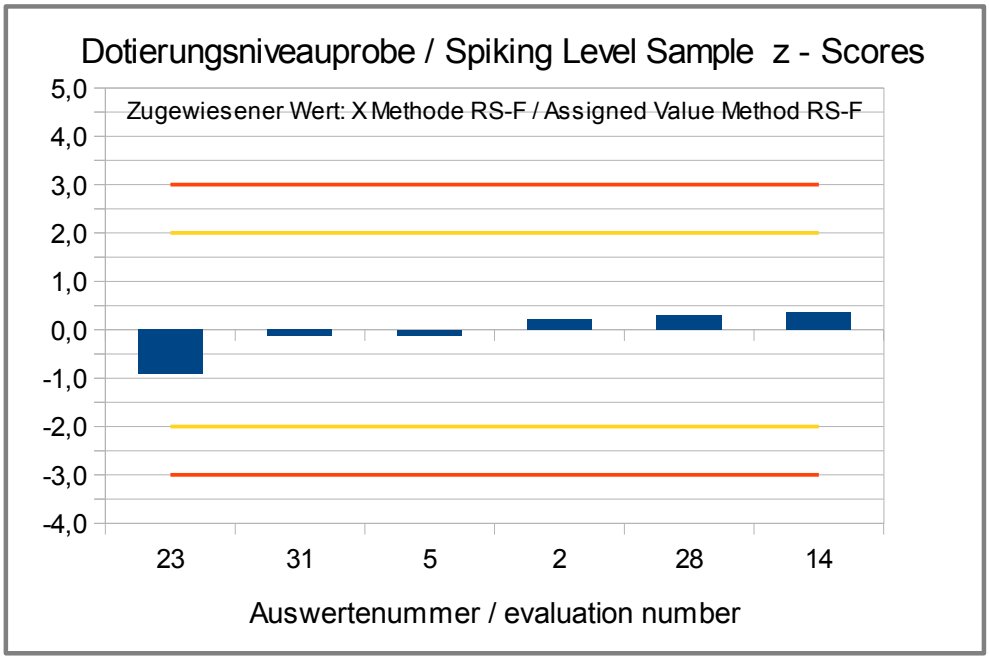


Abb./Fig. 11:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen)

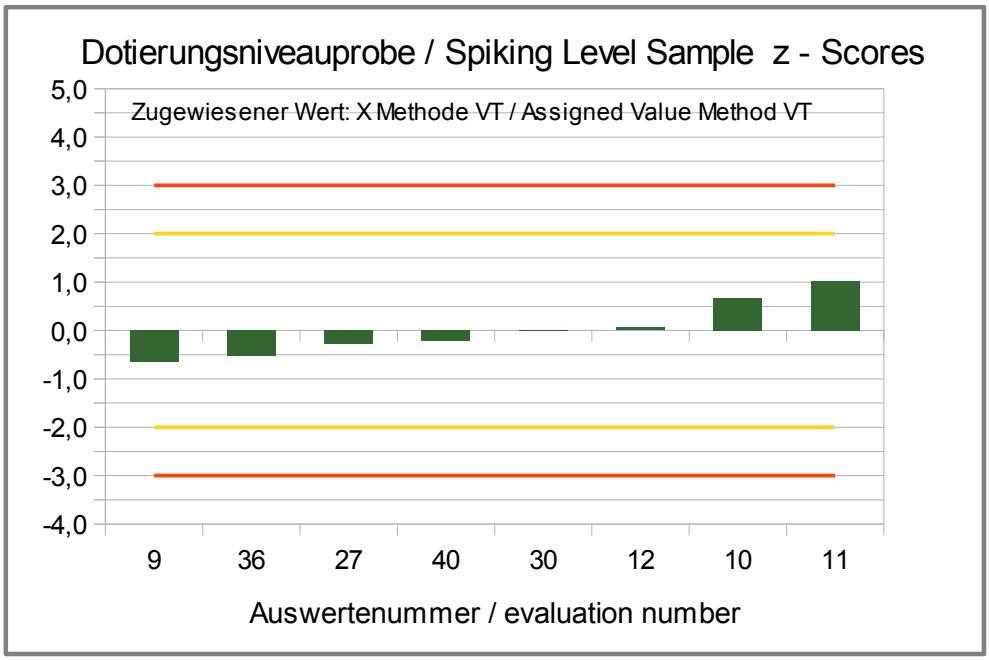


Abb./Fig. 12:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

Recovery Rates ELISA for Mustard: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	166	282	48,4	**80**	AQ	
7	> 40		> 40		AQ	
33	151	256	42,9	**71**	AQ	
29	101	171	89,6	**149**	BC	
13	87,9	**149**	93,5	155	BF	
8	68,6	**117**	49,0	**81**	ES	result converted °
38	125	213	100	166	IL	
2	108	184	72,8	**121**	RS-F	
5	99,2	169	56,9	**94**	RS-F	
14	112	190	83,5	**138**	RS-F	
17					RS-F	
20	> 13,5		> 13,5		RS-F	
23	79,0	**134**	83,0	**138**	RS-F	
28	110	187	110	183	RS-F	
31	98,9	168	96,0	159	RS-F	
9	84,0	**143**	50,1	**83**	VT	
10	118	200	94,0	156	VT	
11	126	214	109	181	VT	
12	102	173	82,0	**136**	VT	
18			267	443	VT	result converted °
27	93,7	159	110	182	VT	
30	100	170	66,0	**109**	VT	
36	87,0	**148**	38,0	**63**	VT	
40	95,0	162	75,0	**124**	VT	

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**5**	Number in RA	**13**
Percent in RA	**25**	Percent in RA	**62**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For the spiking level sample 25% (5) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 62% (13) of the obtained recovery rates were within the recommended range.

4.2.2 PCR Results: Mustard (Sinapis alba)

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	negative	< 31	positive	> 400	2/2 (100%)	ASU	as mustard, white (as brown or black mustard: sample A <4,7 mg/kg)
15	negative		positive	66,6	2/2 (100%)	ASU	
24	negative		positive	114	2/2 (100%)	ASU	
28	negative		positive		2/2 (100%)	ASU	
7	negative		positive		2/2 (100%)	GI	
32	negative		positive	30,0	2/2 (100%)	MS	
2	negative	< 1,0	positive	17,8	2/2 (100%)	SFA	
26	negative		positive		2/2 (100%)	SFA-4p	
8	negative	< 0,4	positive	> 0,4	2/2 (100%)	SFA-ID	
12	negative	< 1	positive	29,8	2/2 (100%)	SFA-ID	
22	negative		positive		2/2 (100%)	SFA-ID	
19	negative		positive		2/2 (100%)	div	
21	negative		positive	120	2/2 (100%)	div	
25	negative		positive		2/2 (100%)	div	
30	negative		positive		2/2 (100%)	div	
34	negative		positive		2/2 (100%)	div	
37	negative		positive		2/2 (100%)	div	
35a	negative		positive		2/2 (100%)	div	
35b	negative		negative		1/2 (50%)	div	as mustard brown and/or black
40a	negative	< 10	negative	< 10	1/2 (50%)	div	as mustard brown
40b	negative	< 100	positive	< 400	2/2 (100%)	div	as mustard yellow

	Sample A		Sample B	
Number positive	0		19	
Number negative	21		2	
Percent positive	0		90	
Percent negative	100		10	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B with yellow mustard (Sinapis alba). Two negative results were obtained for sample B by PCR methods specific for brown and/or black mustard.

Quantitative Valuation PCR: Sample B

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done.

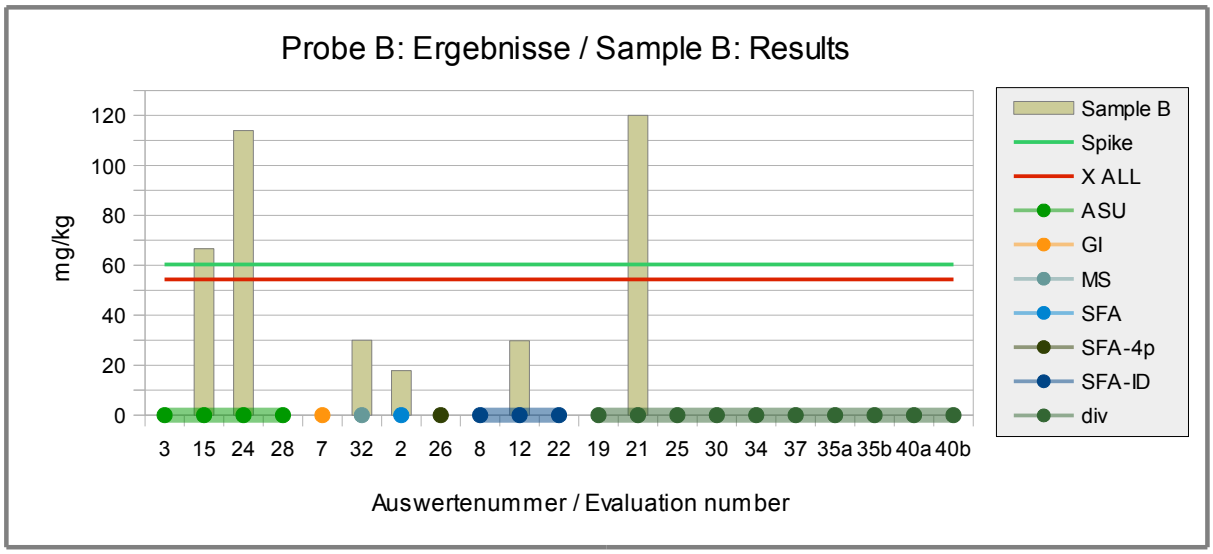


Abb./Fig. 13: PCR Results Mustard (Sample B)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

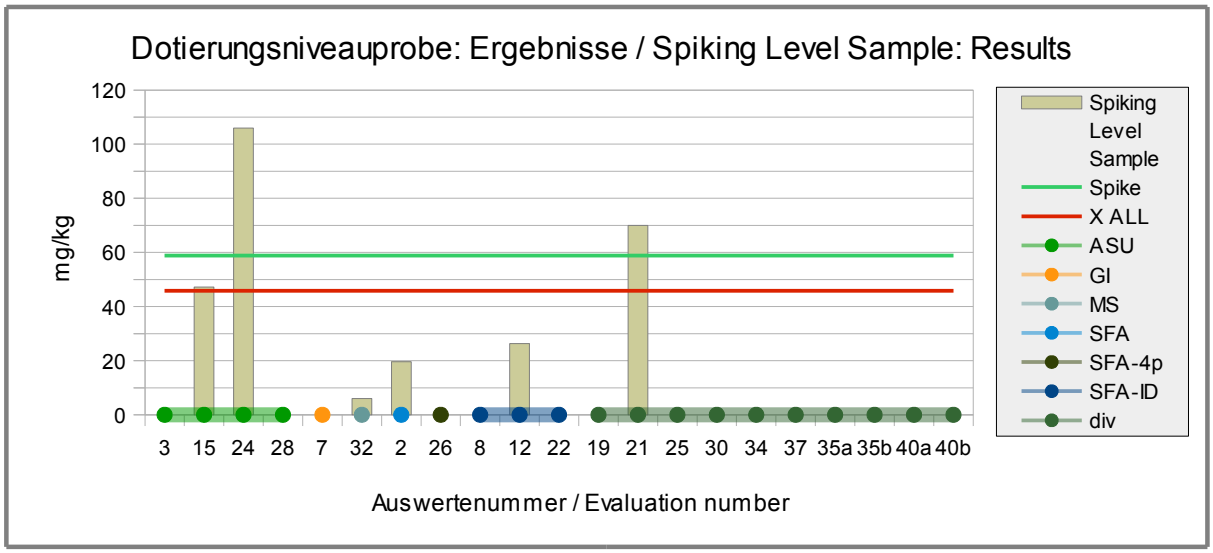


Abb./Fig. 14: PCR Results Mustard (Spiking Level Sample)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done. Moreover the quantitative PCR results for mustard were sometimes unclear or implausible given as mustard DNA.

Evaluation number	Mustard	Mustard	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
3	positive	> 400		ASU	as mustard, white
15	positive	47,2		ASU	as mustard
24	positive	106		ASU	as mustard seed, white
28	positive			ASU	
7	positive			GI	
32	positive	6,00		MS	as mustard DNA
2	positive	19,6		SFA	as mustard DNA
26	positive			SFA-4p	
8	positive	> 0.4		SFA-ID	
12	positive	26,3		SFA-ID	as mustard
22				SFA-ID	
19	positive			div	
21	positive	70,0		div	as mustard DNA, yellow mustard
25	positive			div	
30	positive			div	
34	positive			div	
37	positive			div	
35a	positive			div	
35b	positive			div	as mustard, brown and black
40a	negative	< 10		div	as mustard, brown
40b	positive	< 400		div	as mustard yellow

Number positive	19	
Number negative	1	
Percent positive	95	
Percent negative	5	
Consensus value	positive	

Methods:
ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
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

Recovery Rates PCR for Mustard (informative only): Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3	> 400		> 400		ASU	as mustard, white
15	47,2	80	66,6	110	ASU	as mustard
24	106	180	114	189	ASU	as mustard seed, white
28					ASU	
7					GI	
32	6,00	10	30,0	50	MS	as mustard DNA
2	19,6	33	17,8	30	SFA	as mustard DNA
26					SFA-4p	
8	> 0.4		> 0.4		SFA-ID	
12	26,3	45	29,8	49	SFA-ID	as mustard
22					SFA-ID	
19					div	
21	70,0	119	120	199	div	as mustard DNA, yellow mustard
25					div	
30					div	
34					div	
37					div	
35a					div	
35b					div	as mustard, brown and black
40a	< 10		< 10		div	as mustard, brown
40b	< 400		< 400		div	as mustard yellow

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:

The indication of the recovery rates for mustard by PCR determination is exclusively informative, because on one hand the reference is mustard seed (Sinapis alba) (see p.5) and on the other hand partly other references are indicated for participants' results and partly the reference given is not plausible (as DNA).

4.3 Proficiency Test Sesame

4.3.1 ELISA Results: Sesame

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		
6	negative	< 2	positive	24,5	2/2 (100%)	AQ	
7	negative	0	positive	> 30	2/2 (100%)	AQ	
5	negative	< 2,0	positive	29,1	2/2 (100%)	BC	
29	negative	< 2	positive	21,8	2/2 (100%)	BC	
13	negative	0	positive	35,3	2/2 (100%)	BF	
10	negative	< 2,0	positive	22,5	2/2 (100%)	EF	
30	negative	< 2	positive	26,0	2/2 (100%)	EF	
33	negative	< 2	positive	26,0	2/2 (100%)	EF	
8	negative	< 0,55	positive	30,0	2/2 (100%)	ES	result converted °
18	negative	< LOD	positive	3,52	2/2 (100%)	ES	result converted °
9	negative	< 1.1	positive	37,8	2/2 (100%)	ES-n	result converted °
36	negative	ND	positive	30,9	2/2 (100%)	ES-n	result converted °
38	negative	< 0,5	positive	27,0	2/2 (100%)	IL	
17	negative		positive		2/2 (100%)	NL	
28	negative	< LOQ	positive	27,3	2/2 (100%)	NL-E	
1	negative	< 2,5	positive	89,0	2/2 (100%)	RS-F	
3	negative	< 2,5	positive	89,0	2/2 (100%)	RS-F	
12	negative	< 2,5	positive	64,8	2/2 (100%)	RS-F	
14	negative	< 0,14	positive	78,0	2/2 (100%)	RS-F	
20	negative	< 2,5	positive	> 20	2/2 (100%)	RS-F	
22	negative		positive	58,0	2/2 (100%)	RS-F	
23	negative	< 2,5	positive	120	2/2 (100%)	RS-F	
27	negative	< 2,5	positive	99,8	2/2 (100%)	RS-F	
31	negative		positive	103	2/2 (100%)	RS-F	
39	negative		positive	30,0	2/2 (100%)	RS-F	result converted °
11	negative	< 2,5	positive	165	2/2 (100%)	VT	
16	negative	< 2,5	positive	228	2/2 (100%)	VT	
40	negative	< 2,5	positive	170	2/2 (100%)	VT	

° calculation p. 19

	Sample A		Sample B	
Number positive	0		28	
Number negative	28		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = Eurofins Technologies
ES = ELISA Systems
ES-n = ELISA Systems neu
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of results: Sample B

Evaluation number	Sesame	z-Score Xpt_{ALL30}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
6	24,5	-0,41		AQ	
7	> 30			AQ	
5	29,1	0,26		BC	
29	21,8	-0,81		BC	
13	35,3	1,2		BF	
10	22,5	-0,70		EF	
30	26,0	-0,19		EF	
33	26,0	-0,19		EF	
8	30,0	0,40		ES	result converted °
18	3,52	-3,5		ES	result converted °
9	37,8	1,5		ES-n	result converted °
36	30,9	0,53		ES-n	result converted °
38	27,0	-0,05		IL	
17				NL	
28	27,3	0,00		NL-E	
1	89,0		0,32	RS-F	
3	89,0		0,32	RS-F	
12	64,8		-0,86	RS-F	
14	78,0		-0,22	RS-F	
20	> 20			RS-F	
22	58,0		-1,2	RS-F	
23	120		1,8	RS-F	
27	99,8		0,84	RS-F	
31	103		1,0	RS-F	
39	30,0		-2,5	RS-F	result converted °
11	165			VT	
16	228			VT	
40	170			VT	

° calculation p. 19

Methoden:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = Eurofins Technologies
ES = ELISA Systems
ES-n = ELISA Systems neu
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments: next page

The kernel density estimation (fig. 15) and the figure of the results (fig. 16) show a clear method-dependent distribution of results, so that no joint evaluation of the results from all methods was done. Only a joint evaluation of the results of the methods which account for the main peak at approx. 30 mg/kg was carried out. In addition, a single evaluation was carried out for the methods with at least 5 quantitative results (method RS-F).

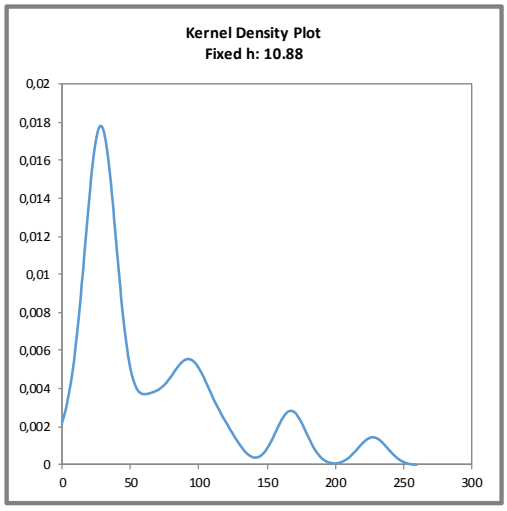


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density plot shows a main peak at approx. 30 mg/kg. Another maximum at approx. 100 mg/kg is due to the results of method RS-F and two smaller side-peaks at 170 and 225 mg/kg are results of the method VT.

Characteristics: Quantitative evaluation ELISA Sesame

Sample B

Statistic Data	**Meth. Peak 30** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL30}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	13	9
Number of outliers	-	-
Mean	26,3	81,3
Median	27,0	89,0
Robust Mean (X_{pt})	**27,3**	**82,5**
Robust standard deviation (S*)	**5,61**	**28,2**
Target range:		
Target standard deviation σ_{pt}	**6,83**	**20,6**
lower limit of target range	**13,7**	**41,2**
upper limit of target range	**41,0**	**124**
Quotient S/σ_{pt}*	*0,82*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*1,95*	*11,8*
Results in the target range	*12*	*8*
Percent in the target range	*92*	*89*

Methods:
Peak 30 = AgraQuant, BioCheck, BioFront Technologies, Eurofins Technologies, ELISA Systems (2 Methoden), Immunolab, Nutrilinia (2 Methoden)
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed clear method-dependent differences. Therefore no joint evaluation of the results of all methods was done. The valuation was done for all results of the main peak ("Peak 30") and separately for method RS-F with more than 5 single results.

The evaluation of results of peak 30 as well as the results of method RS-F showed a normal to low variability of results. The quotients S/σ_{pt} were well below 2,0. The robust standard deviation is in the range of established values for the repeatability and 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.*

The assigned value X_{pt} (robust mean) of peak 30 was 72% of the spiking level of sesame to sample B and thus within the recommendations for the applied methods, while the robust mean of method RS-F was with 218% above this range (s. 3.4.3 and "recovery rates for sesame", p.58).

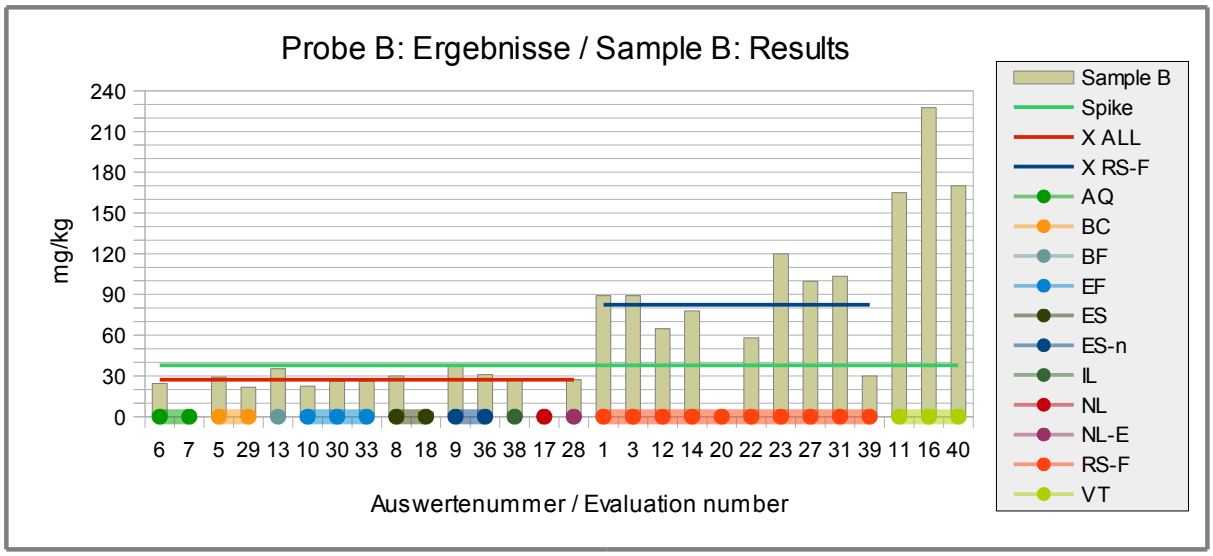


Abb./Fig. 16: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 30"
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

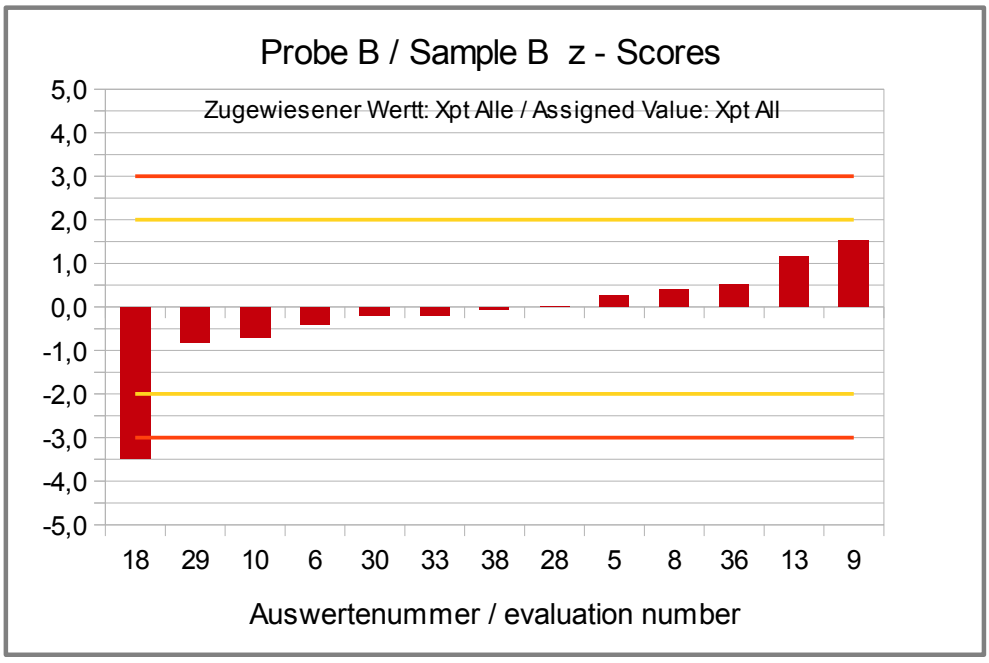


Abb./Fig. 17:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results "peak 30"

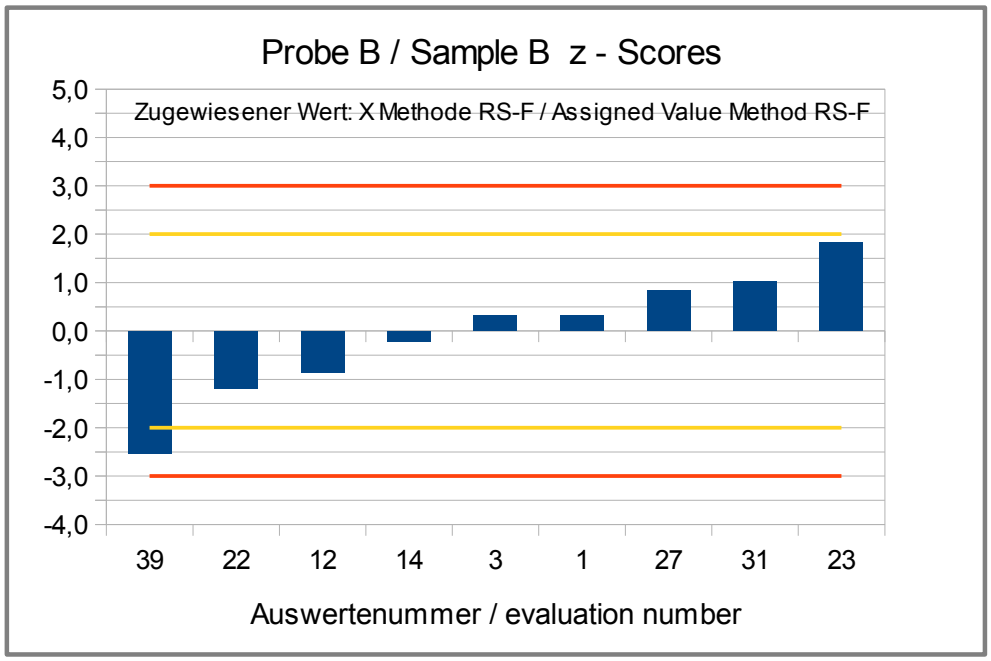


Abb./Fig. 18:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of ELISA: Spiking level sample

Evaluation number	Sesame	z-Score Xpt_{ALL30}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
6	25,1	-1,1		AQ	
7	> 30			AQ	
5	49,2	1,7		BC	
29	28,5	-0,69		BC	
13	62,6	3,3		BF	
10	22,5	-1,4		EF	
30	31,0	-0,40		EF	
33	29,0	-0,63		EF	
8	33,0	-0,16		ES	result converted °
18				ES	result converted °
9	50,2	1,8		ES-n	result converted °
36				ES-n	result converted °
38	29,0	-0,63		IL	
17				NL	
28	29,0	-0,63		NL-E	
1	94,0		-0,27	RS-F	
3	94,0		-0,27	RS-F	
12	115		0,56	RS-F	
14	110		0,36	RS-F	
20	> 20			RS-F	
22	83,5		-0,69	RS-F	
23	120		0,76	RS-F	
27	104		0,13	RS-F	
31	113		0,47	RS-F	
39	30,0		-2,8	RS-F	result converted °
11	189			VT	
16	266			VT	
40	190			VT	

° calculation p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = Eurofins Technologies
ES = ELISA Systems
ES-n = ELISA Systems neu
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments: next page

The kernel density estimation (fig. 19) and the figure of the results (fig. 20) show a clear method-dependent distribution of results, so that no joint evaluation of the results from all methods was done. Only a joint evaluation of the results of the methods which account for the main peak at approx. 30 mg/kg was carried out. In addition, a single evaluation was carried out for the methods with at least 5 quantitative results (method RS-F).

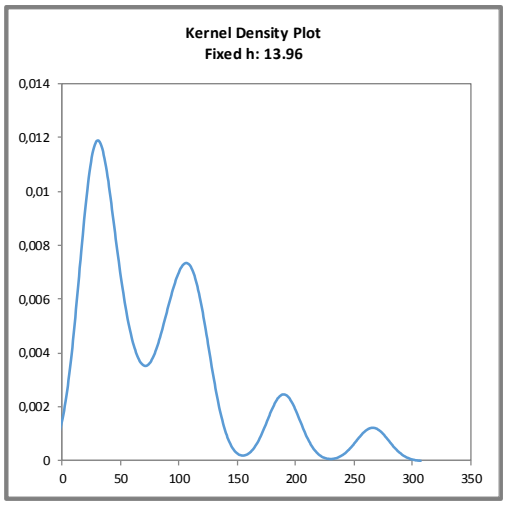


Abb. / Fig. 19:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density plot shows a main peak at approx. 30 mg/kg. Another maximum at approx. 110 mg/kg is due to the results of method RS-F and two smaller side-peaks at 190 and 270 mg/kg are results of the method VT.

Characteristics: Quantitative evaluation ELISA Sesame

Spiking Level Sample

Statistic Data	**Meth. Peak 30** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL30}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	11	9
Number of outliers	-	-
Mean	35,4	96
Median	29,0	104
Robust Mean (X_{pt})	**34,4**	**101**
Robust standard deviation (S*)	**12,2**	**17,4**
Target range:		
Target standard deviation σ_{pt}	**8,61**	**25,2**
lower limit of target range	**17,2**	**50,5**
upper limit of target range	**51,7**	**151**
Quotient S/σ_{pt}*	*1,4*	*0,69*
Standard uncertainty $U_{(Xpt)}$	*4,59*	*7,24*
Results in the target range	*10*	*8*
Percent in the target range	*91*	*89*

Methods:
Peak 30 = AgraQuant, BioCheck, BioFront Technologies, Eurofins Technologies, ELISA Systems (2 Methoden), Immunolab, Nutrilinia (2 Methoden)
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed clear method-dependent differences. Therefore no joint evaluation of the results of all methods was done. The valuation was done for all results of the main peak ("Peak 30") and separately for method RS-F with more than 5 single results.

The evaluation of results of peak 30 as well as the results of method RS-F showed a normal to low variability of results. The quotients S/σ_{pt} were well below 2,0. The robust standard deviation is in the range of established values for the repeatability and 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.*

The assigned value X_{pt} (robust mean) of peak 30 was 94% of the spiking level of sesame to the spiking level sample and thus within the recommendations for the applied methods, while the robust mean of method RS-F was with 274% above this range (s. 3.4.3 and "recovery rates for sesame", p.51).

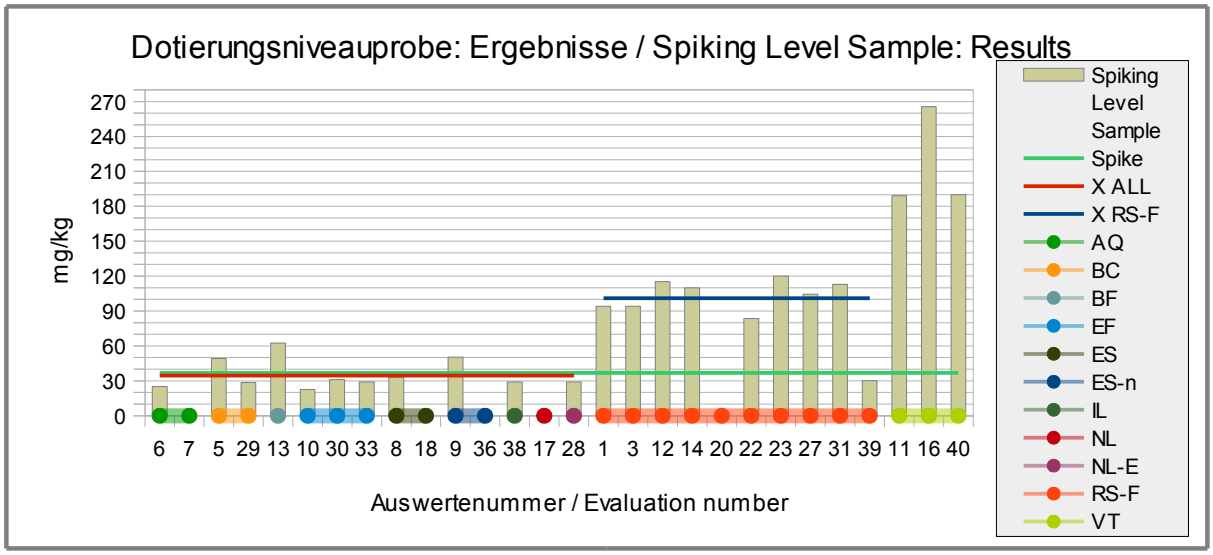


Abb./Fig. 20: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 30"
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

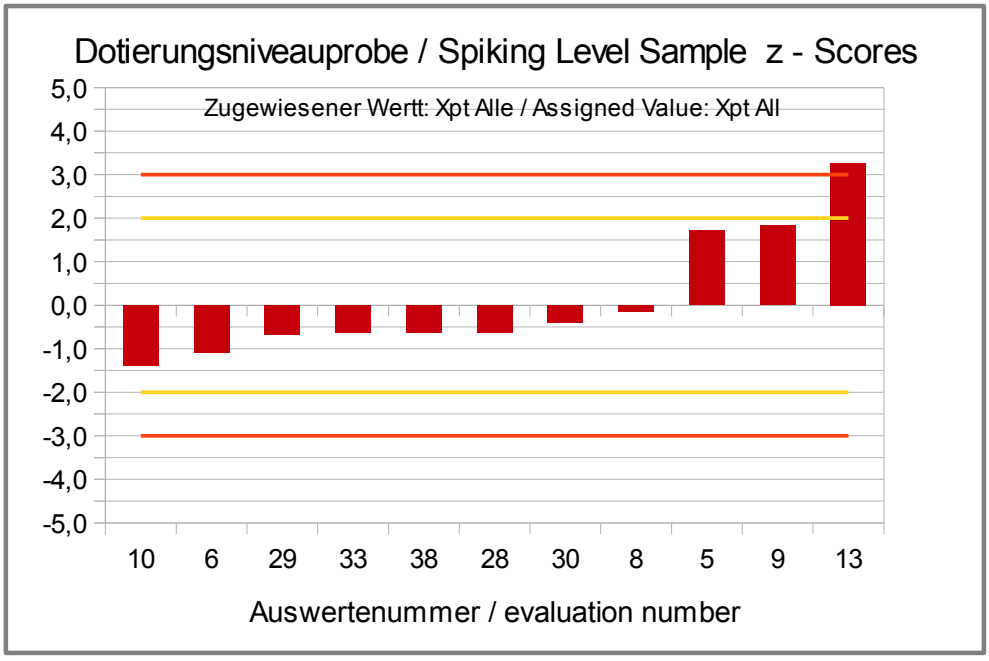


Abb./Fig. 21:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results "peak 30"

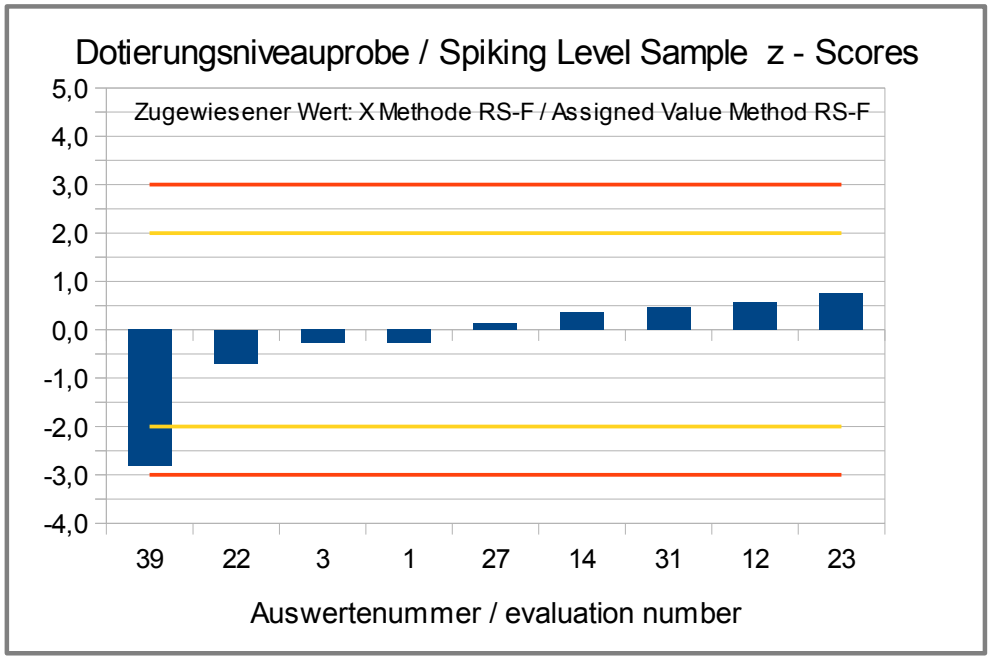


Abb./Fig. 22:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates for Sesame: Spiking level sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	25,1	**68**	24,5	**65**	AQ	
7	> 30		> 30		AQ	
5	49,2	**134**	29,1	**77**	BC	
29	28,5	**77**	21,8	**58**	BC	
13	62,6	170	35,3	**93**	BF	
10	22,5	**61**	22,5	**60**	EF	
30	31,0	**84**	26,0	**69**	EF	
33	29,0	**79**	26,0	**69**	EF	
8	33,0	**90**	30,0	**80**	ES	result converted °
18			3,52	9	ES	result converted °
9	50,2	**136**	37,8	**100**	ES-n	result converted °
36			30,9	**82**	ES-n	result converted °
38	29,0	**79**	27,0	**71**	IL	
17					NL	
28	29,0	**79**	27,3	**72**	NL-E	
1	94,0	255	89,0	236	RS-F	
3	94,0	255	89,0	236	RS-F	
12	115	313	64,8	172	RS-F	
14	110	298	78,0	206	RS-F	
20	> 20		> 20		RS-F	
22	83,5	227	58,0	154	RS-F	
23	120	326	120	318	RS-F	
27	104	283	99,8	264	RS-F	
31	113	306	103	274	RS-F	
39	30,0	**82**	30,0	**80**	RS-F	result converted °
11	189	513	165	437	VT	
16	266	721	228	603	VT	
40	190	516	170	450	VT	

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**13**
Percent in RA	**48**	Percent in RA	**52**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = Eurofins Technologies
ES = ELISA Systems
ES-n = ELISA Systems neu
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
NL-E = nutriLinia®E Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking level sample 48% (11) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B also 52% (13) of the obtained recovery rates were within the recommended range. With one exception the recovery rates of the results of the methods RS-F and VT were above the range of acceptance.

4.3.2 PCR Results: Sesame

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		
24	negative		positive	56,6	2/2 (100%)	ASU	
31	negative		positive		2/2 (100%)	ASU	
7	negative		positive		2/2 (100%)	GI	
32	negative		positive	2,00	2/2 (100%)	MS	
2	negative	< 1,0	positive	7,18	2/2 (100%)	SFA	
8	negative	< 0,4	positive	> 0,4	2/2 (100%)	SFA-ID	
15	negative		positive	> 10	2/2 (100%)	SFA-ID	
22	negative		positive		2/2 (100%)	SFA-ID	
26	negative		positive		2/2 (100%)	SFA-ID	
34	negative		positive		2/2 (100%)	SFA-ID	
19	negative		negative		1/2 (50%)	div	
21	negative		positive	30,0	2/2 (100%)	div	
25	negative		positive		2/2 (100%)	div	
28	negative		positive		2/2 (100%)	div	
30	negative		positive		2/2 (100%)	div	
30	negative		positive		2/2 (100%)	div	
35	negative		positive		2/2 (100%)	div	
37	negative		positive		2/2 (100%)	div	
40	negative	< 100	positive	100	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		18	
Number negative	19		1	
Percent positive	0		95	
Percent negative	100		5	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B. One negative results was obtained for sample B.

Quantitative Valuation PCR: Sample B

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done.

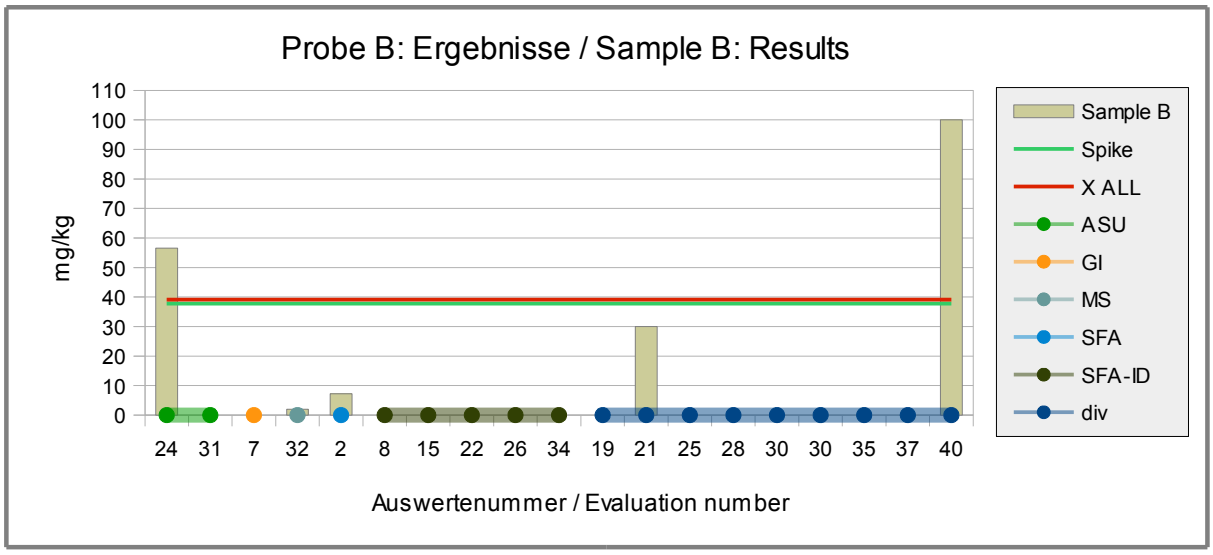


Abb./Fig. 23: PCR Results Sesame (Sample B)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

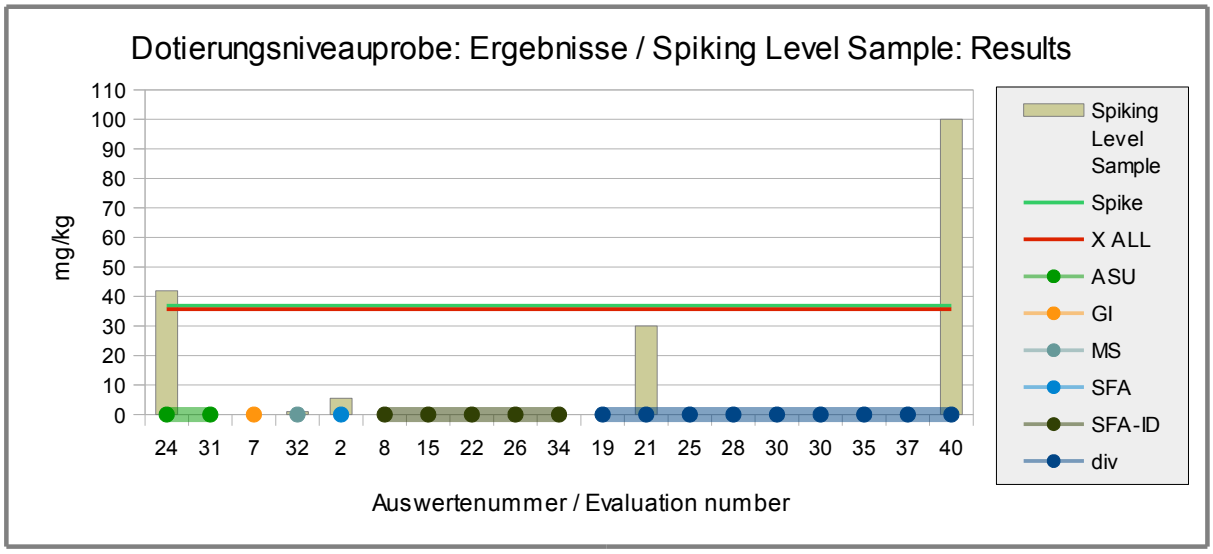


Abb./Fig. 24: PCR Results Sesame (Spiking Level Sample)
green line = Spiking level
red line = robust mean all results (informative)
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Comments:
Due to the high variability and the low number of results, no statistical evaluation was done. Moreover the quantitative PCR results for sesame were sometimes unclear or implausible given as sesame DNA.

Evaluation number	Sesame	Sesame	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
24	positive	41,9		ASU	as sesame
31	positive			ASU	
7	negative			GI	
32	positive	1,00		MS	as sesame DNA
2	positive	5,50		SFA	as sesame DNA
8	positive	> 0,4		SFA-ID	as sesame
15	positive	> 10		SFA-ID	as sesame
22				SFA-ID	
26	positive			SFA-ID	
34	positive			SFA-ID	
19	positive			div	
21	positive	30,0		div	as sesame DNA, sesame seed
25	positive			div	
28	positive			div	
30	positive			div	
30	positive			div	
35	positive			div	
37	positive			div	
40	positive	100		div	as sesame

Number positive	17	
Number negative	1	
Percent positive	94	
Percent negative	6	
Consensus value	positive	

Methods:
ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Recovery Rates PCR for Sesame (informative only): Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
24	41,9	114	56,6	150	ASU	as sesame
31					ASU	
7					GI	
32	1,00	2,7	2,00	5	MS	as sesame DNA
2	5,50	15	7,18	19	SFA	as sesame DNA
8	> 0,4		> 0,4		SFA-ID	as sesame
15	> 10		> 10		SFA-ID	as sesame
22					SFA-ID	
26					SFA-ID	
34					SFA-ID	
19					div	
21	30,0	81	30	79	div	as sesame DNA, sesame seed
25					div	
28					div	
30					div	
30					div	
35					div	
37					div	
40	100	272	100	265	div	as sesame

RA**	50-150 %	RA**	50-150 %
Number in RA	2	Number in RA	2
Percent in RA	40	Percent in RA	40

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:

The indication of the recovery rates for sesame by PCR determination is exclusively informative, because on one hand the reference is sesame seed (see p.5) and on the other hand partly other references are indicated for participants' results and partly the reference given is not plausible (as DNA).

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: Mustard

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	%	e.g. food /protein	Test-Kit + Manufacturer
AQ	6	02.01.00	negative	<2	positive	48,4	positive	166,1		2	44,8	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	7	04.07.18	negative	0	positive	>40	positive	>40	1	2	15	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	33	22/06/18	negative	<2	positive	42,9	positive	150,6	0,2	2	40	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
BC	29	26.06.18	negative	<2	positive	89,6	positive	100,8	2	2	50	Mustard	BioCheck ELISA Mustard-Check
BF	13	07.08.18	negative	0	positive	93,5	positive	87,9	0,13	1		Mustard	MonoTrace Mustard ELISA kit, BioFront Technologies
ES	8	10.08.18	negative	< 1	positive	15	positive	21	1	3		Mustardprotein	ELISA Systems Mustard ESMUS-48
IL	38		negative	<1.2	positive	100	positive	125	1	2		Mustard	Immunolab Mustard ELISA
RS-F	2	18.06.	negative	< 0,5	positive	72,8	positive	108	0,1	1		Mustard	Ridascreen® FAST Mustard, R6152, R-Biopharm
RS-F	5	20/06-10/08/2018	-	<0.5	-	56,9	-	99,2	0,5	1	30,94	Mustard Seed	R Biopharm FAST Mustard R6152
RS-F	14	10/7-11/7/18	negative	<0,1	positive	83,5	positive	111,8	0,1	1		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	17		negative		positive		positive						Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	20	11.07.18	negative	<0,5	positive	>13,5	positive	>13,5		1		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	23	28.06.18	negative	<0.5	positive	83	positive	79	0,5	1	40	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	28	25.06.18/ 26.06.18	-	< LOQ	-	110,05	-	110,15	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	31	26.06.18	negative		positive	96	positive	98,9	0,5	1	42	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
VT	9	08.08.18	negative	<2.5	positive	50,1	positive	84		3		Mustard	Veratox Mustard, Neogen
VT	10	20.06.18	negative	<2,5	positive	94	positive	117,5	1	3	50	Mustard	Veratox Mustard, Neogen
VT	11	10.07.18	-	<2,5	-	109	-	126				Mustard	Veratox Mustard, Neogen
VT	12	09.08.18	negative	<2.5	positive	82	positive	102	2,5	3	30,2	Mustard	Veratox Mustard, Neogen
VT	18	06.07.18	negative	<LOD	positive	81,8	-	not tested	1	3		Mustardprotein	Veratox Mustard, Neogen
VT	27	06.07.18	negative	<2,5	positive	109,7	positive	93,7		3		Mustard	Veratox Mustard, Neogen
VT	30	20.06.18	negative	<2,5	positive	66	positive	100	1,5	3		Mustard	Veratox Mustard, Neogen
VT	36	19.06.18	negative	ND	positive	38	positive	87	2,5	3	23	Mustard	Veratox Mustard, Neogen
VT	40	11.7./21.08.	negative	<2.5	positive	75	positive	95	2,5	3		Mustard	Veratox Mustard, Neogen

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

Continuation ELISA Mustard:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6			Yes	
AQ	7	against mustard protein		yes	Enzyme immunoassay (ELISA) for the quantitative analysis of mustard in food. Limit of detection: 1 ppm; Limit of quantification: 2 ppm; Measurement range: 2-60 ppm; Cross reactivity to: rape(seed) 15,5%, cabbage(seed) 29,2%, radish(seed) 31,2%, coriander 0,012%, caraway 0,0012%, horseradish 0,0007%, garden cress(seed) 1,5%, cardamon 0,006%, cumin 0,0003%.
AQ	33			Yes	
BC	29	Polyclonal	0.5g sample, PBS buffer extraction at 60°C		
BF	13	Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	no	
ES	8			yes	
IL	38			yes	
RS-F	2		as Per Kit Instructions	yes	
RS-F	5	unknown	1g + 20ml	Yes	
RS-F	14	Mustard	According to Manual	no	
RS-F	17	white, yellow , brown, black mustard	as Per Kit Instructions	yes	
RS-F	20			no	
RS-F	23	The antibody specifically detects white, yellow , brown and black mustard.	As per kit instructions	no	
RS-F	28	specific antibodies against all mustard varieties	as Per Kit Instructions	yes	
RS-F	31		according to handbook	yes	
VT	9				
VT	10		According to kit instructions.	yes	
VT	11		extraction solution: PBS	no	elisa Robonik
VT	12	As Per Kit Instructions	As Per Kit Instructions	No	
VT	18		Extraction:60C pre-heated TRIS 1X extraction buffer / 15 min @ 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	Yes	
VT	27		as is	no	
VT	30	Protein from white, black and brown mustard	as Per Kit Instructions	yes	
VT	36	Poly/Mono	TRIS EDTA Solution / 15 mins / 60oC	yes	Single Result
VT	40				

5.1.2 ELISA: Sesame

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	%	e.g. food /protein	Test-Kit + Manufacturer
AQ	6	05.01.00	negative	<2	positive	24,5	positive	25,1		2	54,7	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
AQ	7	04.07.18	negative	0	positive	>30	positive	>30	0,2	2	15	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
BC	5	20/06-10/08/2018	-	<2.0	-	29,1	-	49,2	2	2	43,14	Sesame Seeds	Biocheck (UK) Sesame Check R6029
BC	29	26.06.18	negative	<2	positive	21,8	positive	28,5	2	2	50	Sesame	BioCheck ELISA Sesame-Check
BF	13	07.08.18	negative	0	positive	35,3	positive	62,6	0,3	1		Sesame	MonoTrace Sesame ELISA kit, BioFront Technologies
EF	10	20.06.18	negative	<2,0	positive	22,5	positive	22,5	0,5	2	50	Sesame	Enzyme Immunoassay for the Quantitative Determination of Sesame in Food (Cat. No. HU0030022) Eurofins Technologies
EF	30	18.06.18	negative	<2	positive	26	positive	31	1,5	2		Sesame	Eurofins Technologies Test-Combination HU0030022:2
EF	33	22/06/18	negative	<2	positive	26	positive	29	0,2	2	48	Sesame	Selection Sesame-Kits:
ES	8	10.08.18	negative	< 0.125	positive	7	positive	7,7	0,125	1		Sesameprotein	ELISA Systems Sesame ESSESRD-48
ES	18	03.08.18	negative	<LOD	positive	0,82	-	not tested	0,25	1		Sesameprotein	ELISA Systems Sesame ESSESRD-48
ES-n	9	08.08.18	negative	<0.25	positive	8,8	positive	11,7		0		Sesameprotein	Elisa Systems ESSESE-48
ES-n	36	19.06.18	negative	ND	positive	7,2	-	NA	0,25	0	29	Sesameprotein	ELISA Systems Sesame ESSESE-48
IL	38		negative	< 0.5	positive	27	positive	29	0.2	2		Sesame	Immunolab Sesame ELISA
NL	17		negative		positive		positive						NutriLinia NC-6005-48 Romer Lab
NL-E	28	18.06.18/ 19.06.18	-	< BG	-	27,3	-	29,02	0,2	2		Sesame	nutriLinia Sesam -E, NC-6005/96, Romer Labs
RS-F	1	08.08.18	negative	<2.5	positive	89	positive	94	0,2	2,5	13	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	3	09.08.18	negative	< 2,5	positive	89	positive	94	2,5	5	15,6	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	12	15.08.18	negative	<2.5	positive	64,8	positive	115,1	2,5	3	29,38	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	14	26/7-31/7/18	negative	<0,14	positive	77,95	positive	109,9	0,14	3		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	20	18.06.18	negative	<2,5	positive	>20	positive	>20		3		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	22		negative		positive	58	positive	83,45	0,14	3		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	23	26.06.18	negative	<2.5	positive	120	positive	120	2,5	3	40	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	27	04.07.18	negative	<2.5	positive	99,8	positive	104,3		3		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	31	28.06.18	negative		positive	103,4	positive	112,8	2,5	3	20	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	39	28.08.18	negative		positive	7,0	positive	7,0		0	54	Sesameprotein	Ridascreen® FAST Sesame R7202, R-Biopharm
VT	11	06.07.18	-	<2,5	-	165	-	189				Sesame	Veratox Sesame Allergen, Neogen
VT	16	24.07.18	negative	<2.5	positive	227,6	positive	265,6		3		Sesame	Veratox Sesame Allergen, Neogen
VT	40	11.7./31.8.	negative	<2.5	positive	170	positive	190	2,5	3		Sesame	Veratox Sesame Allergen, Neogen

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

Continuation ELISA Sesame:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6			Yes	
AQ	7	against sesame protein		yes	Enzyme immunoassay (ELISA) for the quantitative analysis of sesame in food. Limit of detection: 0,2 ppm; Limit of quantification: 2 ppm; Measurement range: 2-30 ppm, Cross reactivity to: oats 0,0003%, chia seeds 0,36%, bean 0,0003%, cayenne 0,0006%, onion 0,0007%, sunflow er seeds 0,0003%, black sesame 30%.
BC	5	unknow n	1g + 20ml	Yes	
BC	29	Polyclonal	0.5g sample, PBS buffer extraction at 60°C		
BF	13	Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	no	
EF	10		According to kit instructions.	yes	Kit w e use w as not in the drop-dow n.
EF	30	Sesame proteins	As per kit instructions	yes	
EF	33			Yes	Kit - SensiSpec ELISA sesame kit HU0030022
ES	8			yes	
ES	18	2S-albumin	in shaking w aterbath /	Yes	
ES-n	9				
ES-n	36	Polyclonal/ Monoclonal	Extraction Solution Concentrate / 15 mins / 60oC	yes	Single Result
IL	38				
NL	17	Sesame seed	As per kit instructions	yes	
NL-E	28	antibodies against sesame proteins	As per kit instructions	yes	
RS-F	1	Sesame protein	Samples extracted in AEP-SMP extraction buffer, 60C, shaking (150rpm), 10 minutes (at 60C). Centrifuge at 2500g, 10 mins.	Yes	
RS-F	3	Sesame protein	As per kit instructions	yes	
RS-F	12	As per kit instructions	As per kit instructions Ridascreen FAST Sesame	Yes	
RS-F	14	Sesame	According to Manual	no	
RS-F	20			no	
RS-F	22				
RS-F	23	The antibody specifically detects proteins from w hite, black and yellow sesame.	As per kit instructions	no	
RS-F	27		ext buffer + milk pow der / 10 / 60 °C	no	
RS-F	31		according to handbook	yes	
RS-F	39		Buffer w ith SMP / 10min / 60°C		Result is expessed as soluble sesame protein. (Protein x 7.3%)
VT	11		extraction solution: PBS	no	elisa Robonik
VT	16		as mentionned in test kit instruction	yes	
VT	40				

5.1.3 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	%	e.g. food /protein	Test-Kit + Manufacturer
ASU	15		negative		positive	61,92	positive	18,84	10	25	50	Celery	ASU §64 Methode/method
ASU	24		negative		positive	38,7	positive	30	5	10	50	Celery seed, dried	ASU §64 Methode/method
ASU	28	27.06.18	negative		positive		positive					Celery	iQ™ Supermix, Biorad Primer/Sonde:Eurofins ASU L 08.00-56 August 2014
ASU	31	06.07.18	negative		positive		positive					Celery-DNA	§64 LFGB L 08.00-56
ASU	34		negative		positive		positive		10			Celery-DNA	ASU §64 Methode/method
ASU	37		negative		positive		positive						§64 LMBG L08.00-56
FP	4		negative		positive		positive	6,47				Celery-DNA	foodproof Detection Kit, BIOTECON Diagnostics
GI	7		negative		positive		negative					Celery	GEN-IAL First Allergen, Coring System Diagnostix
MS	32		negative		positive	170	positive	70	10	100	200	Celery-DNA	Microsynth
SFA	2	25.06.	negative	< 1,0	positive	8,75	positive	7,88	0,4	1		Celery-DNA	SureFood® ALLERGEN Celery, S3605, R-Biopharm/Congen
SFA-4p	26	21.06.18	negative		positive		positive		0,4	1	30	Celery-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	8	10.08.18	negative	< 0.4	positive	> 0.4	positive	> 0.4	0,4			Celery	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	12	17.07.18	negative	<1	positive	119,14	positive	62,23	1	1	32,15	Celery	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	22		negative		positive		-		0,4			Celery-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	23	25.06.18	negative		positive		positive		0,4			Celery-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	14	13/7-6/8/18	negative	<0,4	positive	1,6	positive	1,68	0,4	1		Celery	Sure Food Allergen Quant, R-Biopharm / Congen
div	5	20/06-10/08/2018	neg		pos		pos					Celery Seed DNA	IN-house developed
div	6	08.01.00	negative		positive		positive		10			Celery-DNA	other: In house method
div	17		-		positive		-						
div	19		negative		positive		positive						other
div	21		negative		positive	15	positive	10	10	100		Celery-DNA, Celery tuber	AllAllA; Köppel et al, Two tetraplex real-time PCR for the detection and quantification of DNA from eigth allergens in food; Eur. Food Res. Technol. 230 (2010)
div	25	25th July 2018	negative		positive		positive		20			Please select!	other: in house method
div	30	16.06.18	negative		positive		positive		20			Celery-DNA	
div	35	19.06.18	negative	./.	positive	./.	positive	./.	5	./.	./.	Please select!	CEN/TS 15634-2
div	40	25.7./10.08.	negative	<50	positive	100	positive	<100	50	100		Celery	

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

Continuation PCR Celery:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	15		Extraction by Sure Prep Advanced Fa. Congen; no Clean-Up of extracts	yes	Screening according to § 64 L 08.00-65; confirmation w ith Single-PCR (§64 L 08.00-56)
ASU	24	MDH Gene (101bp)	CTAB-precipitation method, s. e.g. ASU L 18.00-22	yes	calibration/quantification by matrix standards, spiked material: celery seed
ASU	28	Protein of Mannitoldehydrogenase	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 Cycles		
ASU	31		Extraction w ith Maxw ell FFS Kit	yes	
ASU	34	Mannitol-Dehydrogenase	CTAB precipitation, QIAgen PCR Purification Kit, Real Time PCR		
ASU	37	part of Manitholdehydrogenase-Gene	2g sample, Silica column, RealTime-PCR, 45 Cycles	yes	
FP	4		real time PCR	no	
GI	7			yes	Real-time PCR-based method for the detection of specific DNA sequences of celery. The detection limit <5 DNA copies.
MS	32		Wizard Promega	yes	Dotierung zu tief für uns
SFA	2		Extraction according to manual w ith SureFood® PREP Advanced, Protocol 1	yes	
SFA-4p	26		SureFood Prep Advanced Protocol 1	yes	Article no. S3401
SFA-ID	8				
SFA-ID	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA-ID	22				
SFA-ID	23	Not specified in kit	As per kit instructions	no	
SFA-Q	14	Celery	Real time PCR	no	
div	5	mtd	Tris & column extraction, real-time PCR analysis.	Y	
div	6		Gel Electrophoresis	Yes	
div	17			yes	in-house real-time PCR control of contract lab
div	19			yes	
div	21	Mannitol Dehydrogenase	CTAB-Wizard Extraction Real-Time-PCR / Taqman Sonden; 45 Cycles, CTAB-Wizard Extraction, Real-Time-PCR / Taqman Probes; 45 Cycles	yes	LOD/LOQ difficult to indicate, its strongly matrix dependent. Approximate values.
div	25		DNA extraction w ith Biotecon foodproof Sample Preparation kit III		
div	30		CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR, 45 Cycles	yes	§64 LFGB L08.00-56
div	35	Manitol déshydrogenase	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	no	
div	40	Celery			Comparision to 400 ppm rice standard; sample specific LOD and LOQ

5.1.4 PCR: Mustard

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	%	e.g. food /protein	Test-Kit + Manufacturer
ASU	3	09.08.18	negative	bl/br: <4,7; w: <31	positive	w: > 400	positive	w: > 400	bl/br: 4,7; w: 31	bl/br: 9,4; w: 154	bl/br: 3,0; w: 3,0	Mustard	ASU §64 Methode/method
ASU	15		negative		positive	66,6	positive	47,16	5	10	50	Mustard	ASU §64 Methode/method
ASU	24		negative		positive	114	positive	106	5	10	50	other: Mustard seed, white	ASU §64 Methode/method
ASU	28	03.07.18	negative		positive		positive					Mustard	5xQuantiFast® Pathogen PCR Fa.Qiagen Primer/Sonde:'Eurofins § 64 LFGB L 08.00.59 Januar 2013
GI	7		negative		positive		positive					Mustard	GEN-IAL First Allergen, Coring System Diagnostix
MS	32		negative		positive	30	positive	6	10	100	200	Mustard-DNA	Microsynth
SFA	2	21.06.	negative	< 1,0	positive	17,8	positive	19,6	0,4	1		Mustard-DNA	SureFood® ALLERGEN Mustard, S3609, R-Biopharm/Congen
SFA-4p	26	21.06.18	negative		positive		positive		0,4	1	30	Mustard-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	8	10.08.18	negative	< 0.4	positive	> 0.4	positive	> 0.4	0,4			Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	12	17.07.18	negative	<1	positive	29,8	positive	26,31	1	1	32,24	Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	22		negative		positive		-		0,4			Mustard-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	19		negative		positive		positive					Please select!	other
div	21		negative		positive	120	positive	70	20	100		Mustard-DNA, yellow Mustard	Fuchs et al, Development and Validation of a Real-Time PCR Method for the detection of White Mustard in Foods, J. Agric. Food Chem.58 (2010)
div	25	25th July 2018	negative		positive		positive		30			Please select!	other: in house method
div	30	16.06.18	negative		positive		positive		40			Mustard-DNA	Auswahl PCR-Methoden
div	34		negative		positive		positive		0,4			Mustard-DNA	Mustorp et al. 2008 Eur Food Res Technol. 226: 771-778
div	37		negative		positive		positive						Hausmethode
div	35a	25.06.18	negative	./.	positive	./.	positive	./.	5	./.	./.	Please select!	Fuchs M., Cichna-Markl M., Hochegger, R – Development and validation of a real-time PCR method for the detection of w hite mustard (Sinapis alba) in foods. J. Agric. Food Chemis. 2010, 58, 11193-11200.
div	35b	25.06.18	negative	./.	negative	./.	positive	./.	./.	./.	./.	Please select!	Palle-Reisch et al. - Development and validation of a real-time PCR methode for the simultaneous detection of black mustard (Brassica nigra) and brow n mustard (Brassica juncea) - Food Chemistry 138 (2013) 348-355
div	40a	07.08.18	negative	<10	negative	<10	negative	<10	10			Mustard	Auswahl PCR-Methoden
div	40b	25.07.18	negative	<100	positive	<400	positive	<400	100	400		Mustard	Auswahl PCR-Methoden

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

Continuation PCR Mustard:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	3	see §64 L 08.00-65 and §64 L 08.00-59 Primer: MADS F/R/Sample and 11-F/R/Sample	DNA-Extraction with NucleoSpin Food 250 units (Macherery-Nagel), Proteinase K + RNAse, Quantstudio 5 Thermo Fisher Scientific, Determination of Concentration by Nano-Drop Thermo Fisher Scientific, Cycler: 45	yes	blackr/ brown (bl/br) and white (w) mustard separately indicated. Sample A,B as well as spiking sample were bl/br negative.
ASU	15		Extraction with Sure Prep Advanced Fa. Congen; no Clean-Up of extracts	yes	Screening and quantitative determination by § 64 L 08.00-65; confirmation of qualitative result by Single-PCR (§64 L 08.00-64 (black and brown mustard) and SureFood Allergen ID Fa. Congen (yellow mustard))
ASU	24	MADS D (74bp)	CTAB-precipitation method, s. e.g. ASU L 18.00-22	yes	calibration/quantification by matrix standards, spiked material: mustard seed, white
ASU	28	MADS-D-Protein of Sinapis alba	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 Cycles		
GI	7			yes	Real-time PCR-based method for the detection of specific DNA sequences of yellow, brown and black mustard in food. The detection limit <5 DNA copies.
MS	32		Wizard Promega	yes	spiking too low for us
SFA	2		Extraction according to manual with SureFood® PREP Advanced, Protocol 1	yes	
SFA-4p	26		SureFood Prep Advanced Protocol 1	yes	Article no. S3401
SFA-ID	8				
SFA-ID	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA-ID	22				
div	19			no	
div	21	MADS D Gene	see above	yes	see above
div	25		DNA extraction with Biotecon foodproof Sample Preparation kit III		
div	30		CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR, 45 Cycles	yes	§64 LFGB L08.00-65:2017
div	34	major allergen sin a1	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR		
div	37	Sinapis alba/ Brassica nigra/ B. juncea	2g sample, Silica column, RealTime-PCR, 45 Cycles	yes	
div	35a	MADS-D	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	no	Sinapis alba
div	35b	Partial RT gene for reverse transcriptase from gypsy-like retroelement 13G42-26	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 43 cycles	no	
div	40a	mustard, brown			Comparision to 400 ppm rice standard; sample specific LOD and LOQ
div	40b	mustard, yellow			Comparision to 400 ppm rice standard; sample specific LOD and LOQ

5.1.5 PCR: Sesame

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	%	e.g. food /protein	Test-Kit + Manufacturer
ASU	24		negative		positive	56,6	positive	41,9	5	10	50	Sesame	ASU §64 Methode/method
ASU	31	06.07.18	negative		positive		positive					Sesame-DNA	ASU L 18.00-22
GI	7		negative		positive		negative					Sesame	GEN-IAL First Allergen, Coring System Diagnostix
MS	32		negative		positive	2	positive	1	10	100	200	Sesame-DNA	Microsynth
SFA	2	22.06.	negative	< 1,0	positive	7,18	positive	5,5	0,4	1		Sesame-DNA	SureFood® ALLERGEN Sesame, S3608, R-Biopharm/Congen
SFA-ID	8	10.08.18	negative	< 0.4	positive	> 0.4	positive	> 0.4	0,4			Sesame	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	15		negative		positive	> 10	positive	> 10	10			Sesame	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	22		negative		positive		-		0,4			Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	26	21.06.18	negative		positive		positive		0,4	1	30	Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	34		negative		positive		positive		0,4			Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	19		negative		negative		positive					Please select!	other
div	21		negative		positive	30	positive	30	10	100		Sesame-DNA, Sesame seed	AIAIIB; Köppel et al, siehe Sellerie
div	25	25th July 2018	negative		positive		positive		25			Please select!	other: in house method
div	28	03.07.18	negative		positive		positive					Sesame	5xQuantiFast® Pathogen PCR Fa.Qiagen Primer/Sonde: Eurofins Methode nach Mustorp et al. 2007
div	30	16.06.18	negative		positive		positive		20			Sesame-DNA	Auswahl PCR-Methoden
div	30	16.06.18	negative		positive		positive		0,4			Mustard-DNA	Auswahl PCR-Methoden
div	35	25.06.18	negative	./.	positive	./.	positive	./.	5	./.	./.	Please select!	Waiblinger H-U - Ring trial validation of single and multiplexx real-time PCR methods for the detection and quantification of the allerginic food ingredients sesame, almond, lupine and Brazil nur - J. Verbr. Lebensm. - DOI 10,1007/s00003-014-0868-x
div	37		negative		positive		positive						Hausmethode
div	40	25.7./10.08.	negative	<100	positive	100	positive	100	100	100		Sesame	Auswahl PCR-Methoden

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

Continuation PCR Sesame:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	24	2 S Albumin Gene (66bp)	CTAB-precipitation method, s. e.g. ASU L 18.00-22	yes	calibration/quantification by matrix standards, spiked material: sesame, defatted
ASU	31		Extraction with Maxwell FFS Kit	yes	
GI	7			yes	Real-time PCR-based method for the detection of specific DNA sequences of sesame. The detection limit <5 DNA copies.
MS	32		Wizard Promega	yes	spiking too low for us
SFA	2		Extraction according to manual with SureFood® PREP Advanced, Protocol 1	yes	
SFA-ID	8				
SFA-ID	15		Extraction by Sure Prep Advanced Fa. Congen; no Clean-Up of extracts	yes	Analysis only with SureFood Allergen ID, Fa. Congen
SFA-ID	22				
SFA-ID	26		SureFood Prep Advanced Protocol 1	yes	Article no. S3608
SFA-ID	34	Sesame	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR		
div	19			yes	
div	21	15.5 kDa oleosin mRNA	see above	yes	see above
div	25		DNA extraction with Biotecon foodproof Sample Preparation kit III		
div	28	2 S Albumin	Dneasy ®mericon Food Kit/ Proteinase K/ Real Time PCR/ 45 Cycles		
div	30		CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR, 45 Cycles	yes	§64 LFGB L18.00-19
div	30		CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR, 45 Zyklen	yes	Mustard and Brassica varietes DNA, internal method
div	35	Albumine 2S	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	no	
div	37	Sesamum indicum, S. radiacum	2g sample, Silica column, RealTime-PCR, 45 Cycles	yes	
div	40	Sesame			Comparision to 400 ppm rice standard; sample specific LOD and LOQ

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 04-2018 Dotierungsniveauprobe

Weight whole sample	1,04	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	42,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	107	42,7
2	5,03	131	52,1
3	4,98	131	52,6
4	5,03	138	54,9
5	4,99	140	56,1
6	4,97	137	55,1
7	5,05	124	49,1
8	5,10	122	47,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	128,8	Particles
Standard deviation	11,4	Particles
χ^2 (CHI-Quadrat)	7,01	
Probability	**43**	%
Recovery rate	122	%

Normal distribution		
Number of samples	8	
Mean	51,3	mg/kg
Standard deviation	4,53	mg/kg
rel. Standard deviaton	8,82	%
Horwitz standard deviation	8,85	%
HorRat-value	**1,0**	
Recovery rate	122	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter (1st letter):

PT number	***DLA 04-2018***
PT name	***Allergens IV: Celery, Mustard and Sesame in Potato Chips with "Spiking Level Sample“***
Sample matrix (processing)	***Samples A + B:*** *Potato Chips light (fat 22%) / ingredients: Potatoes, sunflower oil, salt and other food additives and allergenic foods celery seeds, mustard and sesame (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods celery seeds, mustard and sesame*
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: Celery, Mustard and Sesame 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. It is the best to homogenize the whole sample.*
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 August 10th 2018***
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. 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
		UNITED KINGDOM
		SWITZERLAND
		Germany
		Germany
		USA
		SWITZERLAND
		Germany
		CANADA
		CANADA
		ITALY
		Germany
		Germany
		Germany
		SPAIN
		Germany
		ZYPRUS
		Germany
		ITALY
		SWEDEN
		UNITED KINGDOM
		FINLAND
		POLAND
		CANADA
		AUSTRIA
		Germany
		POLEN
		SWITZERLAND
		FRANCE
		CANADA
		UNITED KINGDOM
		Germany
		FRANCE
		UNITED KINGDOM
		UNITED KINGDOM
		CROATIA
		CANADA
		SPAIN
		UNITED KINGDOM
		Germany
		CANADA

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen –

Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 18.00-19 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Sesam (Sesamum indicum) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of sesame (Sesamum indicum) in rice and wheat cookies and sauce powders by PCR]
33. ASU §64 LFGB L 18.00-22 Untersuchung von Lebenmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]
34. ASU §64 LFGB L 08.00-59 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
35. ASU §64 LFGB L 08.00-64 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von von schwarzem Senf (Brassica nigra L.) und braunem Senf (Brassica juncea L.) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of black mustard (Brassica nigra L.) and brown mustard (Brassica juncea L.) in boiled sausages by real-time PCR]
36. ASU §64 LFGB L 08.00-65 Untersuchung von Lebenmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR

(2016) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]