Evaluation Report
proficiency test

DLA 04/2019

Allergens IV:

Celery, Mustard and Sesame

in Spice Salt

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

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 14 October 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix sample is a customary iodine salt with addition of commercial spieces (pepper, paprika, onion). The basic composition of samples A and B was the same (see table 1). The ingredients of the basic mixture were mixed and homogenized.

Afterwards the **spiked sample A** was produced as follows:
The spiking materials containing the allergenic ingredients celery, mustard and sesame were crushed and sieved by 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 up to 3 additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned 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 DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Spice Salt, Ingredients: Salt (96%), onion powder (1,5%), pepper (1,3%), paprika (1,1%)	98,4 g/100 g	100 g/100g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	98,5 g/100 g
Celery seed: - as celery* - thereof 20,0% total protein**	 38,9 mg/kg 7,79 mg/kg	-	 35,0 mg/kg 7,01 mg/kg
Mustard, yellow: - as mustard* - thereof 30,6% total protein**	 49,4 mg/kg 15,1 mg/kg	-	 44,5 mg/kg 13,6 mg/kg
Sesame, white: - as Sesame seed* - *thereof 23,3% total protein***	 31,0 mg/kg 7,21 mg/kg	-	 27,9 mg/kg 6,49 mg/kg
further Ingredients: *Maltodextrin, sodium sulfate and silicon dioxide*	<0,02 g/100 g	-	<0,02 g/100 g

* Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw materials (total nitrogen according to Kjeldahl with F=6,25 for celery, mustard and sesame protein)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples A and the spiking level sample showed a probability of 90% and 91%. 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 and 0,8 respectively. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample A by all ELISA tests for mustard (Immunolab, Veratox and AgraQuant) and sesame (Immunolab, Veratox and AgraQuant), respectively (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

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

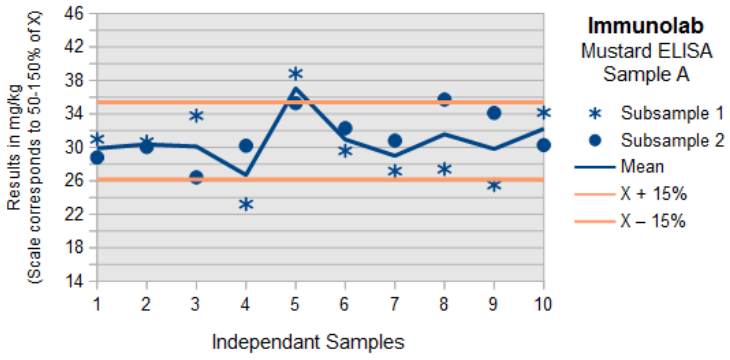
ELISA-Tests: Homogenität Senf / Homogeneity Mustard

Immunolab Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 30,8 ± 1,8 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	31,0	28,8	29,9
2	30,7	30,0	30,4
3	33,8	26,4	30,1
4	23,2	30,2	26,7
5	38,8	35,3	37,0
6	29,6	32,3	30,9
7	27,2	30,8	29,0
8	27,4	35,7	31,6
9	25,5	34,1	29,8
10	34,2	30,3	32,2

General average X	30,8	
SD of sample means Sx	2,67	8,7%
SD w ithin-samples Sw	2,74	8,9%
SD betw een-samples Ss	1,84	6,0%

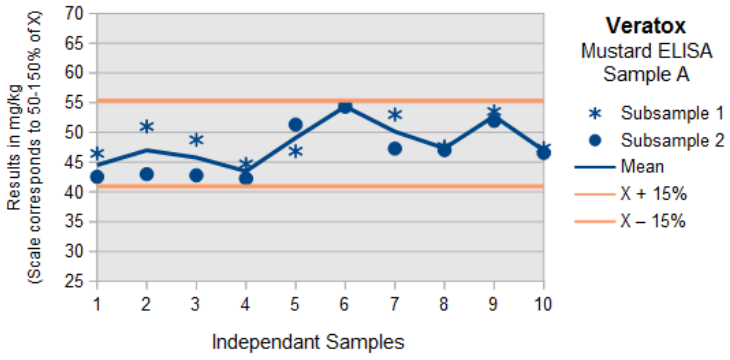


Veratox Mustard ELISA

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Mustard 48,1 ± 3,1 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	46,5	42,5	44,5
2	51,0	43,0	47,0
3	48,8	42,8	45,8
4	44,7	42,2	43,5
5	46,8	51,3	49,1
6	54,4	54,3	54,4
7	53,0	47,3	50,1
8	47,6	47,0	47,3
9	53,5	51,9	52,7
10	47,4	46,6	47,0

General average X	48,1	
SD of sample means Sx	3,47	7,2%
SD w ithin-samples Sw	2,11	4,4%
SD betw een-samples Ss	3,14	6,5%

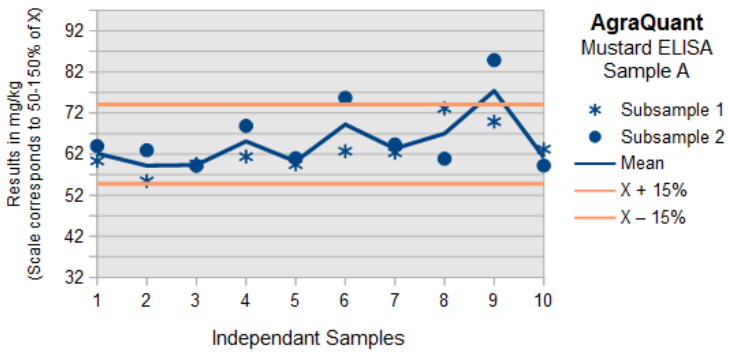


AgraQuant Mustard ELISA

Sample weights: 0,50 g (0,45 – 0,55 g)
Number of replicates: 2
Overall result: Mustard 64,4 ± 4,8 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	60,4	64,0	62,2
2	55,5	62,9	59,2
3	59,6	59,2	59,4
4	61,4	68,9	65,1
5	59,5	61,0	60,2
6	62,7	75,7	69,2
7	62,3	64,4	63,3
8	73,1	60,9	67,0
9	69,9	84,9	77,4
10	63,2	59,2	61,2

General average X	64,4	
SD of sample means Sx	5,63	8,7%
SD w ithin-samples Sw	4,15	6,4%
SD betw een-samples Ss	4,80	7,5%



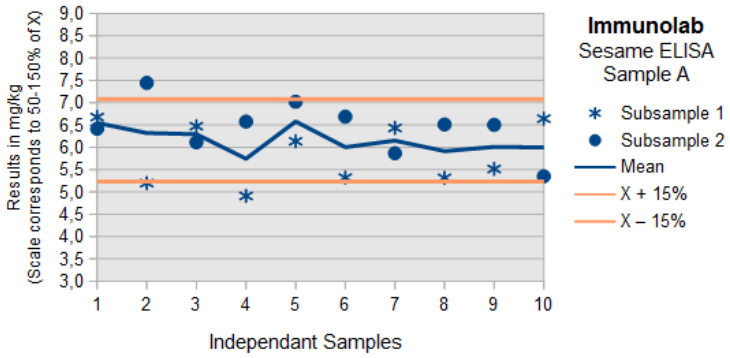
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Immunolab Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 6,16 ± 0,27 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,68	6,42	6,55
2	5,20	7,44	6,32
3	6,47	6,11	6,29
4	4,91	6,58	5,74
5	6,13	7,02	6,58
6	5,32	6,69	6,00
7	6,43	5,87	6,15
8	5,32	6,51	5,91
9	5,52	6,50	6,01
10	6,64	5,35	6,00

General average X	6,16	
SD of sample means Sx	0,27	4,5%
SD w ithin-samples Sw	0,61	10,0%
SD betw een-samples Ss	<0,27	<4,5%

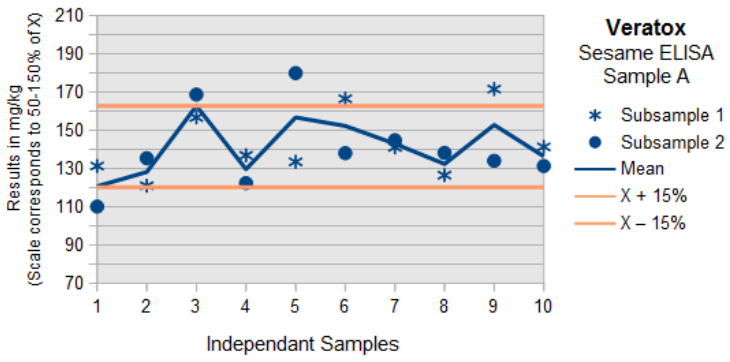


Neogen Veratox ELISA Sesame

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Sesame 141 ± 11 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	131	110	121
2	121	135	128
3	157	169	163
4	137	122	130
5	133	180	157
6	166	138	152
7	141	145	143
8	126	138	132
9	171	134	153
10	141	131	136

General average X	141	
SD of sample means Sx	14,1	10,0%
SD w ithin-samples Sw	11,9	8,4%
SD betw een-samples Ss	11,3	8,0%

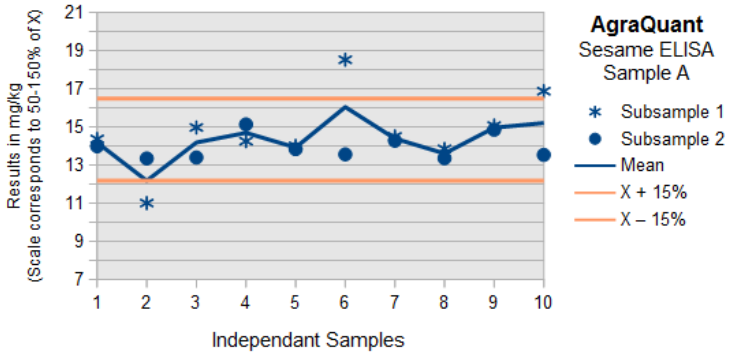


Romerlabs AgraQuant Sesame

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 14,3 ± 0,7 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	14,4	14,0	14,2
2	11,0	13,3	12,2
3	15,0	13,4	14,2
4	14,2	15,1	14,7
5	14,0	13,8	13,9
6	18,5	13,6	16,0
7	14,5	14,3	14,4
8	13,8	13,4	13,6
9	15,1	14,8	15,0
10	16,9	13,5	15,2

General average X	14,3	
SD of sample means Sx	1,03	7,2%
SD w ithin-samples Sw	1,06	7,4%
SD betw een-samples Ss	0,71	5,0%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,39 (21,9°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 23rd week of 2019. The testing method was optional. The tests should be finished at 19th July 2019 the latest.

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

*There are two different samples A and B possibly containing the allergenic parameters **Celery**, **Mustard** and/or **Sesame** in the range of mg/kg in the matrix of **Spice Salt**. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing.*

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

2.3 Submission of results

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

All 42 participants submitted their results on time.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 15 – 43% 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 procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty 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).

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 calculated using z-score or z'-score

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

4.1 Proficiency Test 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		
4	positive		negative		2/2 (100%)	ASU	Sample A: Traces near LOD
13	positive		negative		2/2 (100%)	ASU	
26	positive		negative		2/2 (100%)	ASU	
35	positive		negative		2/2 (100%)	ASU	
23a	positive	0,860	negative		2/2 (100%)	FP	Given as celery DNA
23b	positive		negative		2/2 (100%)	GI	
33	positive		negative		2/2 (100%)	IM	
11	positive		negative		2/2 (100%)	MS	
36	positive	70,0	negative		2/2 (100%)	MS	
10	positive	7,61	positive	1	1/2 (50%)	SFA	
12	positive	28,7	negative	<1	2/2 (100%)	SFA	
17	positive		negative		2/2 (100%)	SFA	
18	positive		positive		1/2 (50%)	SFA	
22	positive		negative		2/2 (100%)	SFA	
29	positive		negative		2/2 (100%)	SFA	
34	positive		positive		1/2 (50%)	SFA	
27	positive		negative		2/2 (100%)	SFA-4p	
1	positive		negative		2/2 (100%)	div	
3	positive		negative	8	2/2 (100%)	div	
8	negative		negative		1/2 (50%)	div	No positive sample identified
14	positive		negative		2/2 (100%)	div	
20	positive		negative		2/2 (100%)	div	
28	positive		negative		2/2 (100%)	div	
38	positive		negative		2/2 (100%)	div	
41	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	24		3	
Number negative	1		22	
Percent positive	96		12	
Percent negative	4		88	
Consensus value	positive		negative	

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GI = GEN-IAL First Allergen
IM = Imegen Celery ID kit
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. Three positive results for sample B were obtained by the method SFA (SureFood Allergen).

Quantitative Valuation PCR: Sample A

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

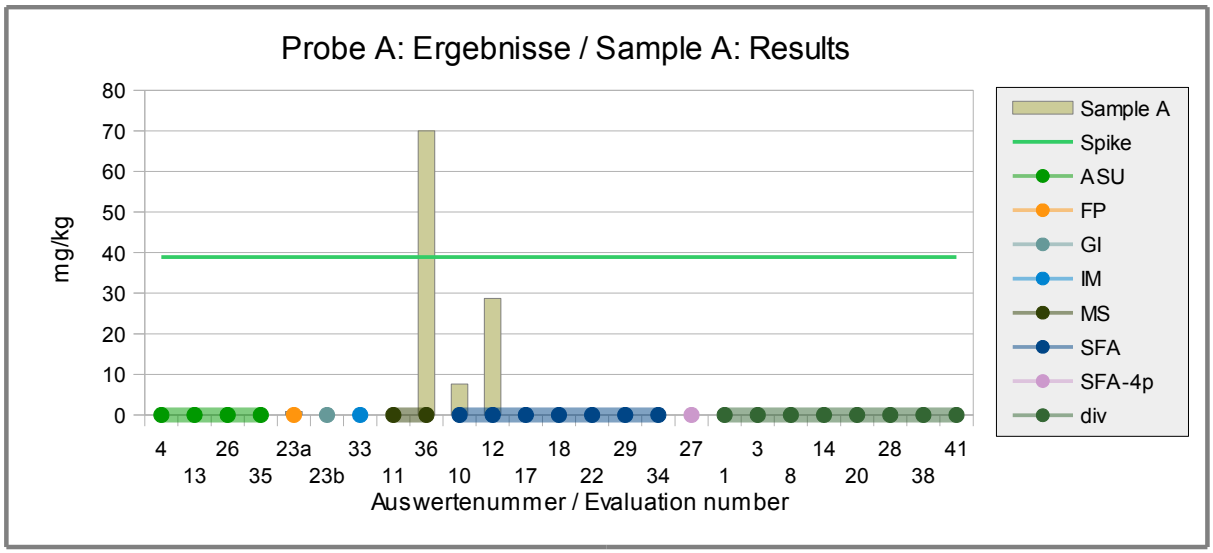


Abb./Fig. 1: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

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

Evaluation number	Celery	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive			ASU	
13	positive			ASU	
26	positive			ASU	
35	positive			ASU	
23a	positive	0,420		FP	Given as celery DNA
23b	positive			GI	
33	positive			IM	
11	positive			MS	
36	positive	100		MS	
10	positive	3,08		SFA	
12	positive	35,2		SFA	
17	positive			SFA	
18	positive			SFA	
22	positive			SFA	
29	positive			SFA	
34	positive			SFA	
27	positive			SFA-4p	
1	positive			div	
3	-			div	
8	positive			div	
14	negative			div	
20	positive			div	
28	positive			div	
38	positive			div	
41	positive			div	

Number positive	23
Number negative	1
Percent positive	96
Percent negative	4
Consensus value	positive

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
IM = Imegen Celery ID kit
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comment:
One negative result was obtained for the spiking level sample.

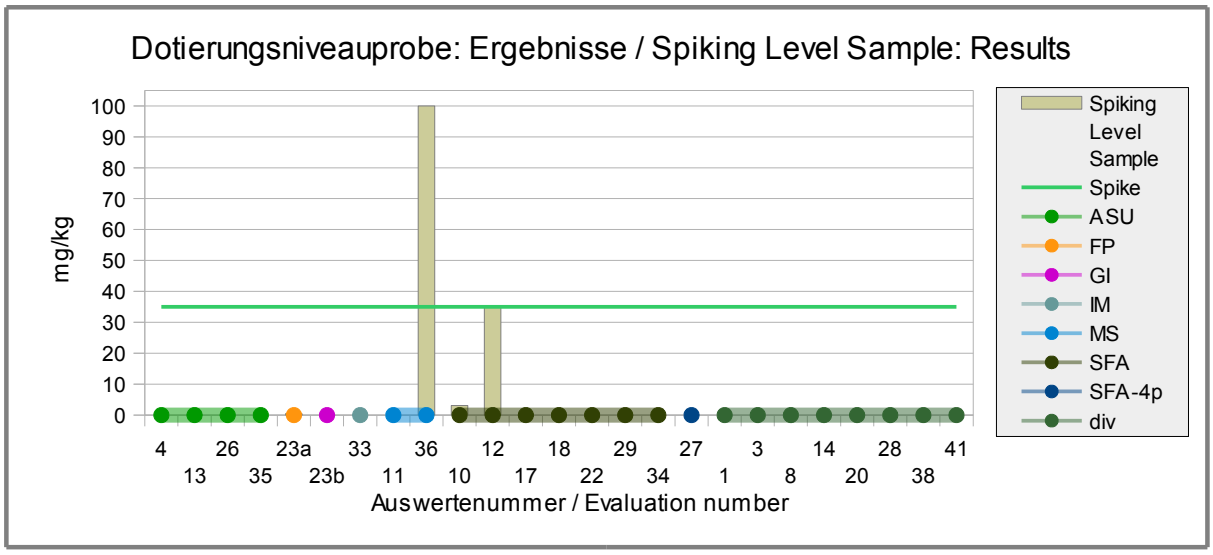


Abb./Fig. 2: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4					ASU	
13					ASU	
26					ASU	
35					ASU	
23a	0,420	(1,2)	0,860	(2,2)	FP	Given as celery DNA (therefore calculated RR questionable)
23b					GI	
33					IM	
11					MS	
36	100	286	70,0	180	MS	
10	3,08	8,8	7,61	20	SFA	
12	35,2	**100**	28,7	**74**	SFA	
17					SFA	
18					SFA	
22					SFA	
29					SFA	
34					SFA	
27					SFA-4p	
1					div	
3					div	
8					div	
14					div	
20					div	
28					div	
38					div	
41					div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
IM = Imegen Celery ID kit
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:

By PCR methods one of 4 participants obtained with both the spiking level sample and the spiked food matrix sample A a recovery rate within the range of the AOAC-recommendation of 50-150%.

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		
14	positive	29,0	negative	<2	2/2 (100%)	AQ	
21	positive	33,8	negative	<2,0	2/2 (100%)	AQ	
23	positive	114	negative	<3,3	2/2 (100%)	AQ	Result converted °
9	positive	25,4	negative	<2	2/2 (100%)	BC	
40	positive	37,0	negative	0	2/2 (100%)	BF	
10a	positive	62,9	negative	< 2,0	2/2 (100%)	EF	
22	positive	63,4	negative	<2	2/2 (100%)	IL	
32	positive	33,6	negative	<0,04	2/2 (100%)	IL	
10b	positive	47,8	negative	< 0,5	2/2 (100%)	RS-F	
12	positive	83,1	negative	<0,5	2/2 (100%)	RS-F	
13	positive	64,2	negative		2/2 (100%)	RS-F	
17	positive	116	negative	<1,6	2/2 (100%)	RS-F	Result converted °
18	positive	69,0	negative	<0,5	2/2 (100%)	RS-F	
19	positive	15,8	negative	0	2/2 (100%)	RS-F	
27	positive		negative		2/2 (100%)	RS-F	
28	positive	69,4	negative	<0,5	2/2 (100%)	RS-F	
31	positive	>13,5	negative	<0,5	2/2 (100%)	RS-F	
2	positive	64,5	negative		2/2 (100%)	VT	
4	positive	56,0	negative	<2,5	2/2 (100%)	VT	
7	positive	64,1	negative	<1,0	2/2 (100%)	VT	
15	positive	42,1	negative		2/2 (100%)	VT	
16	positive	56,2	negative	<2,5	2/2 (100%)	VT	
30	positive	52,0	negative	<2,5	2/2 (100%)	VT	
39	positive	50,5	negative	<2,5	2/2 (100%)	VT	
42	positive	23,5	negative		2/2 (100%)	VT	

° calculation see p. 19

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

Methods:

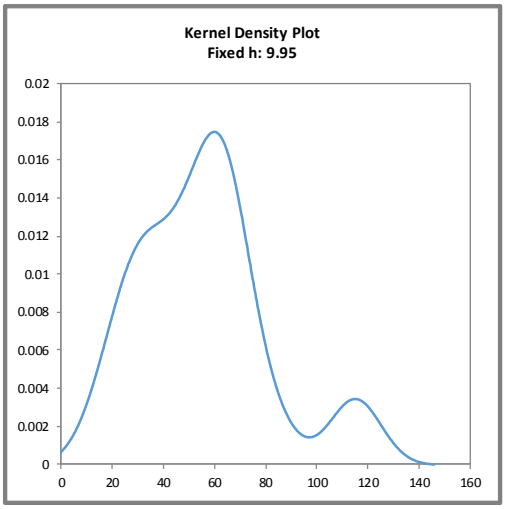
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
14	29,0	-1,8			AQ	
21	33,8	-1,5			AQ	
23	114	4,6			AQ	Result converted °
9	25,4	-2,1			BC	
40	37,0	-1,2			BF	
10a	62,9	0,74			EF	
22	63,4	0,78			IL	
32	33,6	-1,5			IL	
10b	47,8	-0,40	-1,1		RS-F	
12	83,1	2,3	1,0		RS-F	
13	64,2	0,84	-0,14		RS-F	
17	116	4,8	3,0		RS-F	Result converted °
18	69,0	1,2	0,15		RS-F	
19	15,8	-2,8	-3,0		RS-F	
27					RS-F	
28	69,4	1,2	0,17		RS-F	
31	>13,5				RS-F	
2	64,5	0,86		0,90	VT	
4	56,0	0,22		0,26	VT	
7	64,1	0,83		0,87	VT	
15	42,1	-0,83		-0,80	VT	
16	56,2	0,23		0,27	VT	
30	52,0	-0,08		-0,05	VT	
39	50,5	-0,20		-0,16	VT	
42	23,5	-2,2		-2,2	VT	

° calculation see p. 19



Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
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 symmetric distribution of results with a shoulder at approx. 40 mg/kg and a secondary peak at 116 mg/kg, due to increased single values (method RS-F and AQ).

Characteristics: Quantitative evaluation ELISA Mustard

Sample A

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	23	7	8
Number of outliers	0	0	0
Mean	55,4	66,5	51,1
Median	56,0	69,0	54,0
Robust Mean (X_{pt})	**53,1**	**66,5**	**52,6**
Robust standard deviation (S*)	**22,8**	**35,0**	**11,36**
Target range:			
Target standard deviation σ_{pt}	**13,3**	**16,6**	**13,2**
lower limit of target range	**26,5**	**33,3**	**26,3**
upper limit of target range	**79,6**	**100**	**78,9**
Quotient S/σ_{pt}*	*1,7*	*2,1*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*5,96*	*16,5*	*5,02*
Results in the target range	*17*	*5*	*7*
Percent in the target range	*74*	*71*	*88*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed nearly a symmetrical distribution (two increased single results).

The evaluation of all methods and of method VT showed a normal to low variability of results, with quotients S/σpt below 2,0. The evaluation of the results of method RS-F showed a minimally increased variability of results. The quotient S*/σpt was 2,1.*
The robust standard deviations are partly in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 107%, 135% and 107% of the spiking level of Mustard to sample A within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA for Mustard" p.36).

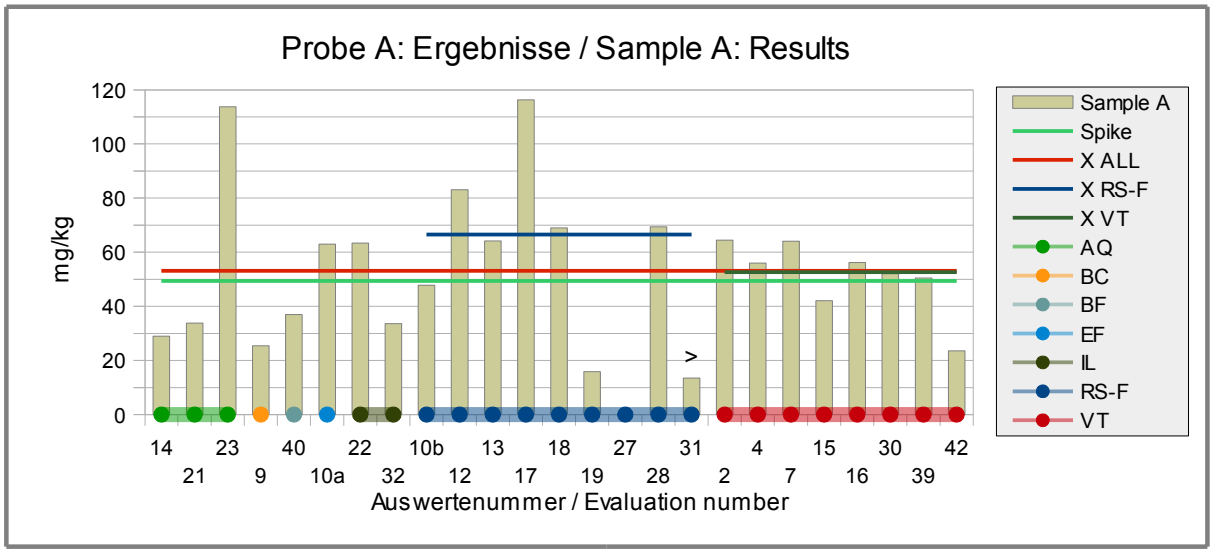


Abb./Fig. 4: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

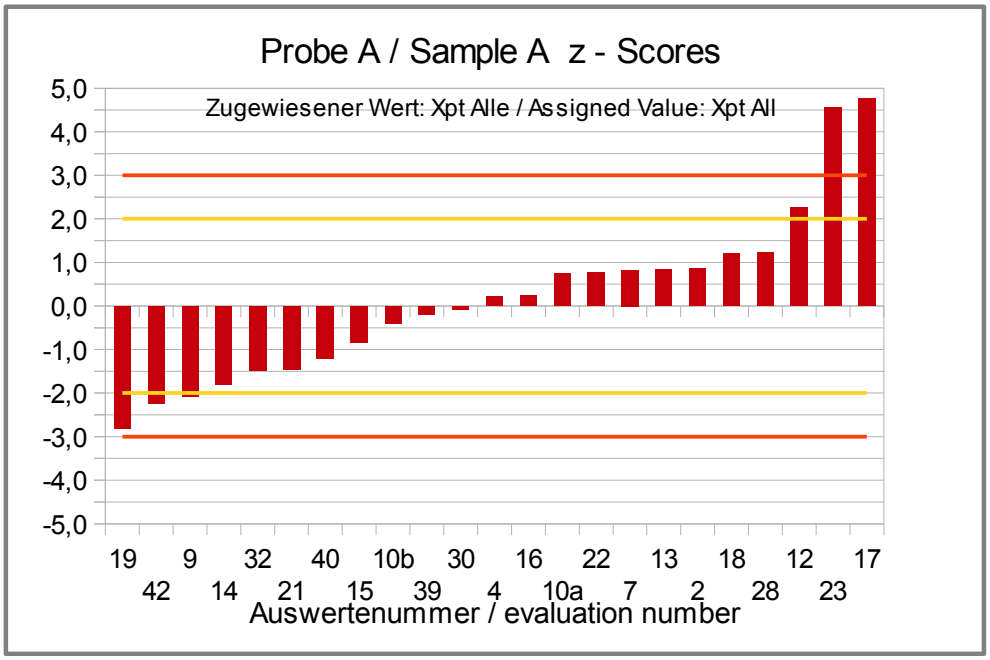


Abb./Fig. 5:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

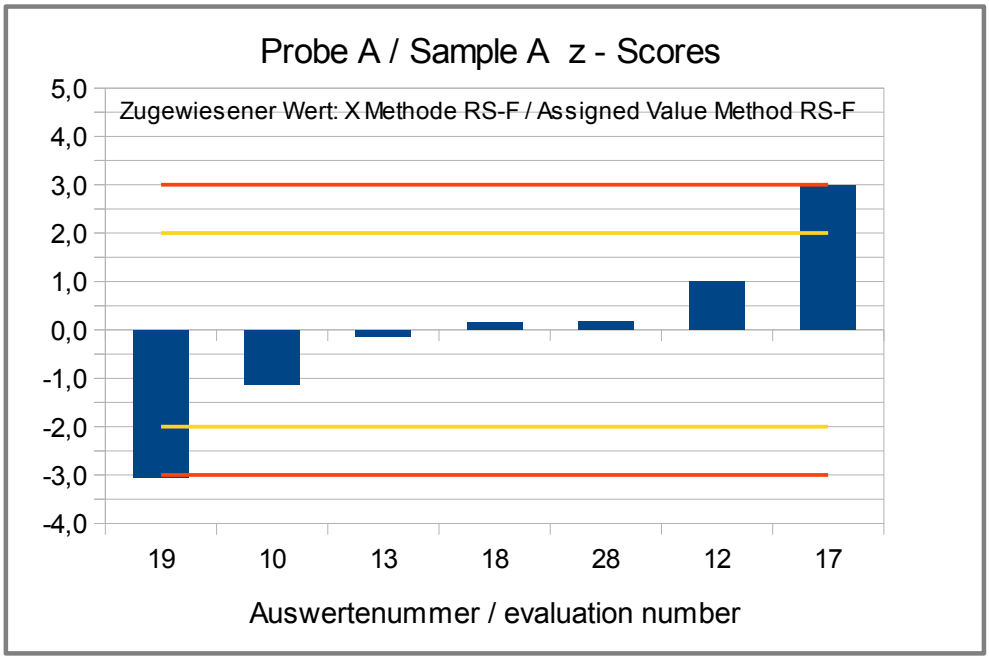


Abb./Fig. 6:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen)

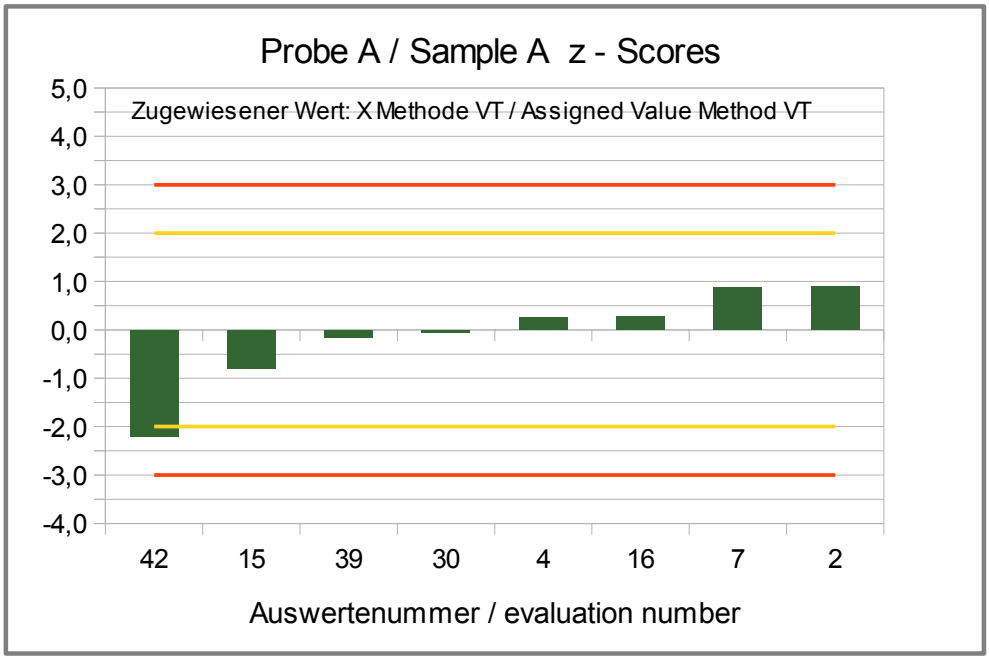
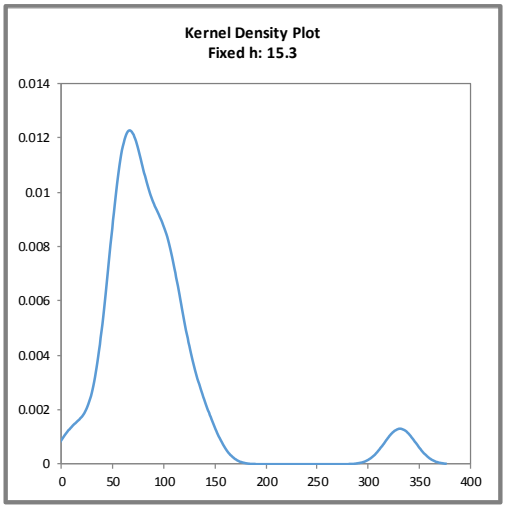


Abb./Fig. 7:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

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]					
14	123	2,0			AQ	
21	139	2,8			AQ	
23	331	12			AQ	Result converted °
9	96,5	0,72			BC	
40	49,0	-1,6			BF	
10a	> 60				EF	
22	99,2	0,85			IL	
32	106	1,2			IL	
10b	>13,5				RS-F	
12	82,3	0,02	1,0		RS-F	
13	72,1	-0,47	0,41		RS-F	
17	64,4	-0,85	-0,06		RS-F	Result converted °
18	65,0	-0,82	-0,02		RS-F	
19	13,2	-3,4	-3,2		RS-F	
27					RS-F	
28	64,4	-0,85	-0,06		RS-F	
31	>13,5				RS-F	
2	102	0,99		1,5	VT	
4	105	1,1		1,6	VT	
7					VT	
15	79,7	-0,10		0,28	VT	
16	52,9	-1,4		-1,2	VT	
30	63,0	-0,9		-0,61	VT	
39	66,0	-0,77		-0,45	VT	
42	52,3	-1,44		-1,19	VT	

° calculation see p. 19



Methods:

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

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

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a shoulder at approx. 100 mg/kg and a secondary peak at about 330 mg/kg, due to a single result above the target range (method AQ).

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	7
Number of outliers	-	-	0
Mean	91,3	60,2	74,4
Median	75,9	64,7	66,0
Robust Mean (X_{pt})	**81,8**	**65,3**	**74,4**
Robust standard deviation (S*)	**31,8**	**14,3**	**24,8**
Target range:			
Target standard deviation σ_{pt}	**20,4**	**16,3**	**18,6**
lower limit of target range	**40,9**	**32,7**	**37,2**
upper limit of target range	**123**	**98,0**	**112**
Quotient S/σ_{pt}*	*1,6*	*0,87*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*8,88*	*7,28*	*11,7*
Results in the target range	*17*	*5*	*7*
Percent in the target range	*85*	*83*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed nearly a symmetrical distribution (a high single value).

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

The robust means of the evaluations were 184%, 147% and 167% of the spiking level of mustard to the spiking level sample and were thus above or in the upper range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA for Mustard" p.36).

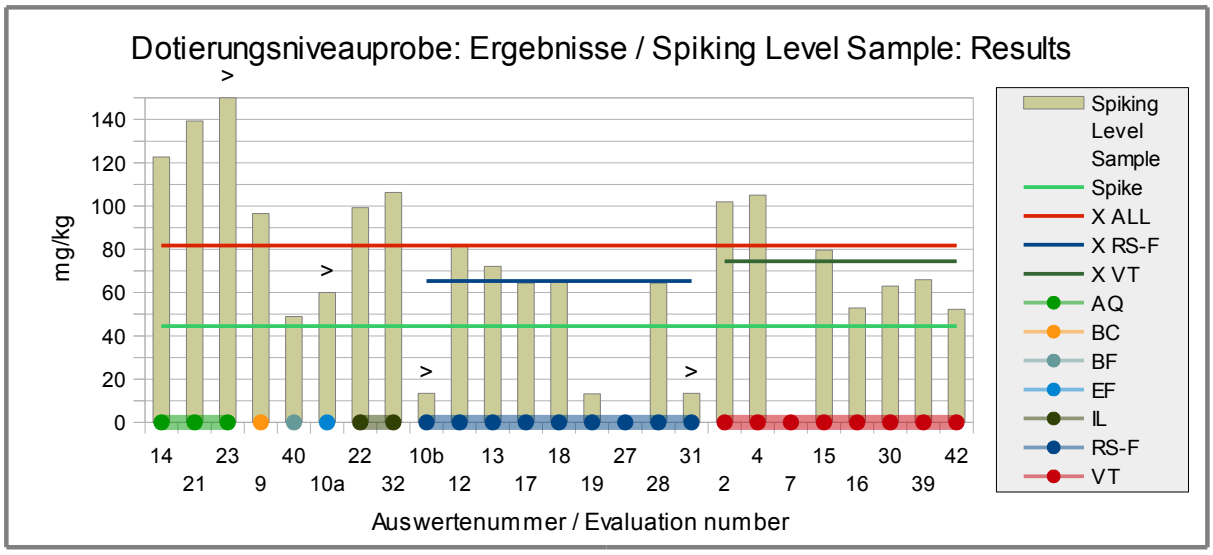


Abb./Fig. 9: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

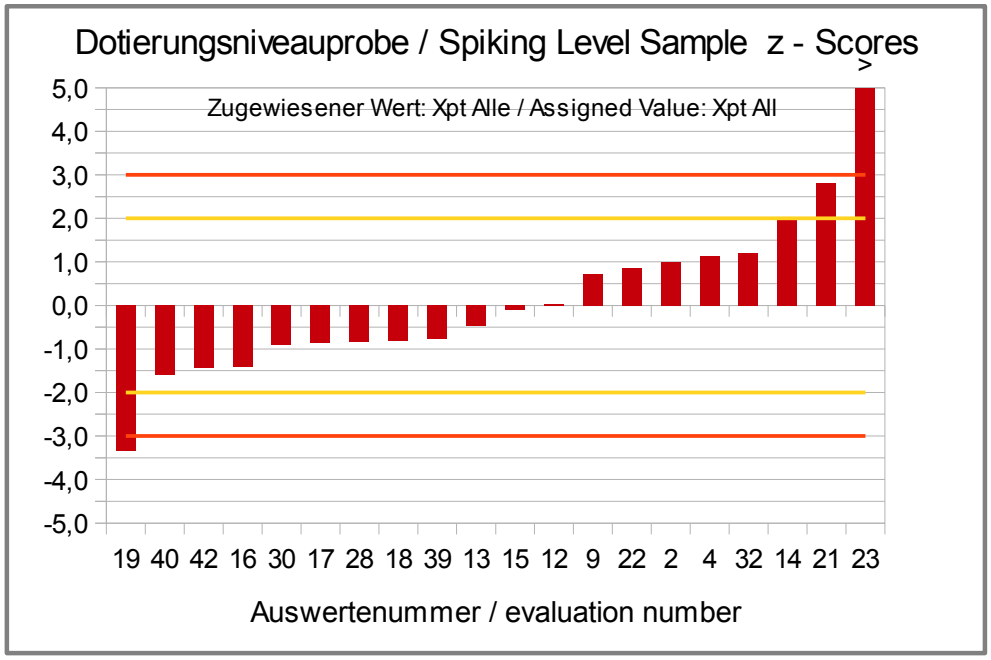


Abb./Fig. 10:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

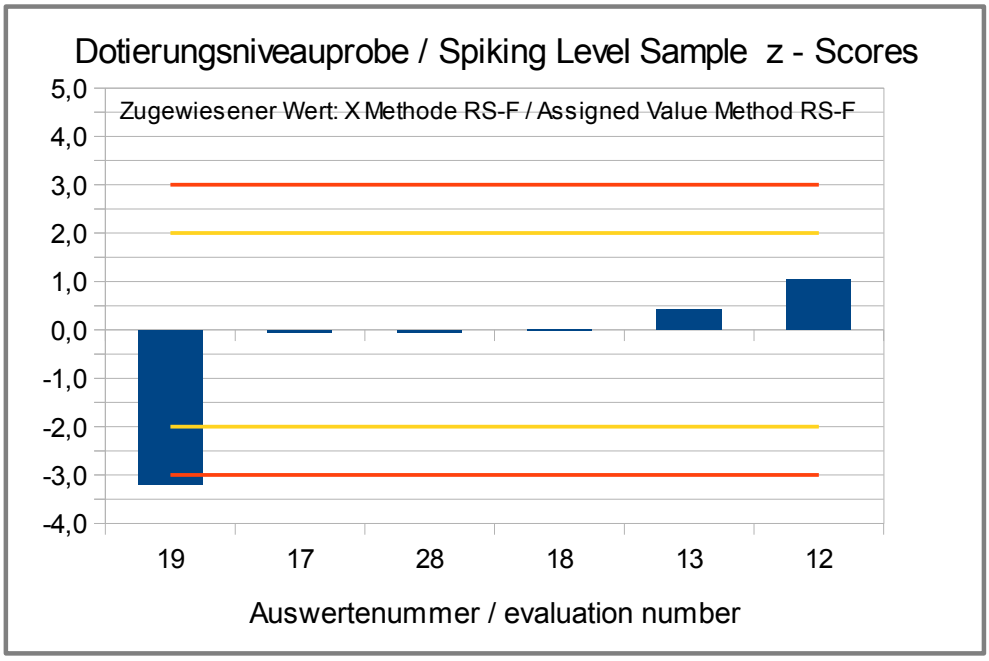


Abb./Fig. 11:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

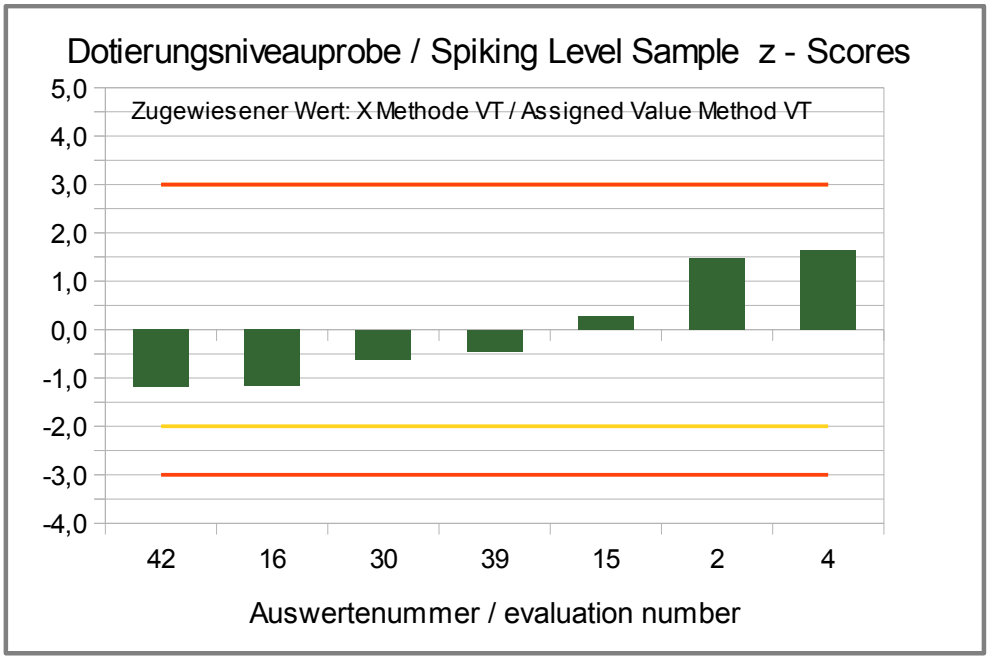


Abb./Fig. 12:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
14	123	276	29,0	**59**	AQ	
21	139	313	33,8	**68**	AQ	
23	331	745	114	230	AQ	Result converted °
9	96,5	217	25,4	**51**	BC	
40	49,0	**110**	37,0	**75**	BF	
10a	>60		62,9	**127**	EF	
22	99,2	223	63,4	**128**	IL	
32	106	239	33,6	**68**	IL	
10b	>13,5		47,8	**97**	RS-F	
12	82,3	185	83,1	168	RS-F	
13	72,1	162	64,2	**130**	RS-F	
17	64,4	**145**	116	236	RS-F	Result converted °
18	65,0	**146**	69,0	**140**	RS-F	
19	13,2	30	15,8	32	RS-F	
27					RS-F	
28	64,4	**145**	69,4	**140**	RS-F	
31	>13,5		>13,5		RS-F	
2	102	229	64,5	**131**	VT	
4	105	236	56,0	**113**	VT	
7			64,1		VT	
15	79,7	179	42,1	**85**	VT	
16	52,9	**119**	56,2	**114**	VT	
30	63,0	**142**	52,0	**105**	VT	
39	66,0	**148**	50,5	**102**	VT	
42	52,3	**118**	23,5	48	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**8**	Number in RA	**17**
Percent in RA	**40**	Percent in RA	**77**

* 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
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

For the spiking level sample 40% (8) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 77% (17) of the recovery rates were within the range of acceptance.

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		
4	positive		negative		2/2 (100%)	ASU	
23	positive		negative		2/2 (100%)	GI	
36	positive	20,0	negative		2/2 (100%)	MS	
12	positive	54,7	negative	<1	2/2 (100%)	SFA	
17	positive		negative		2/2 (100%)	SFA	
29	positive		negative		2/2 (100%)	SFA	
33	positive		negative		2/2 (100%)	SFA	
34	positive		negative		2/2 (100%)	SFA	
27	positive		negative		2/2 (100%)	SFA-4p	
26	positive		negative		2/2 (100%)	SFA-ID	
1a	positive		negative		2/2 (100%)	div	
1b	negative		negative		1/2 (50%)	div	Detection only of brown and black mustard
3	positive		negative	8	2/2 (100%)	div	
8	positive		negative		2/2 (100%)	div	
11	positive		negative		2/2 (100%)	div	
20	positive		negative		2/2 (100%)	div	
35	positive		negative		2/2 (100%)	div	
38	positive		negative		2/2 (100%)	div	
41	positive		negative		2/2 (100%)	div	

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

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 = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:

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

For sample A one negative result was obtained with a method specific for brown and black mustard. However, the sample contains white/yellow mustard.

Quantitative valuation of PCR-results: Sample A

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

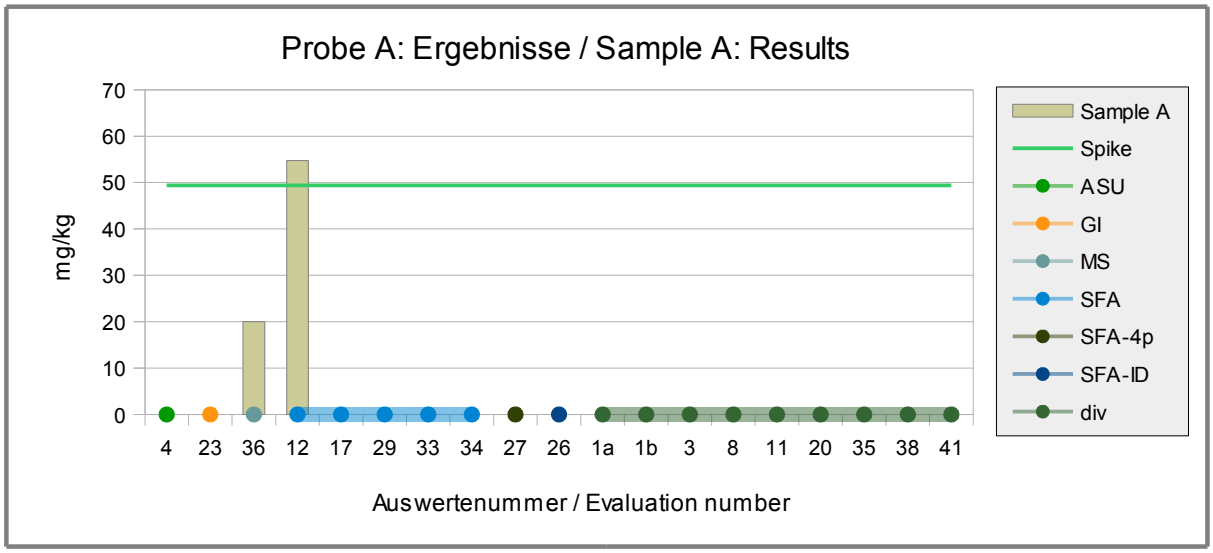


Abb./Fig. 13: PCR Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation of PCR-results: Spiking level sample

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

Evaluation number	Mustard	Mustard	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive			ASU	
23	positive			GI	
36	positive	30,0		MS	
12	positive	110		SFA	
17	positive			SFA	
29	positive			SFA	
33	positive			SFA	
34	positive			SFA	
27	positive			SFA-4p	
26	positive			SFA-ID	
1a	positive			div	
1b	positive (Spuren)			div	Detection only of brow n and black mustard
3				div	
8	positive			div	
11	positive			div	
20	positive			div	
35	positive			div	
38	positive			div	
41	positive			div	

Number positive	17
Number negative	0
Percent positive	100
Percent negative	0
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 = keine genaue Angabe / andere Methode
div = not indicated / other method

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

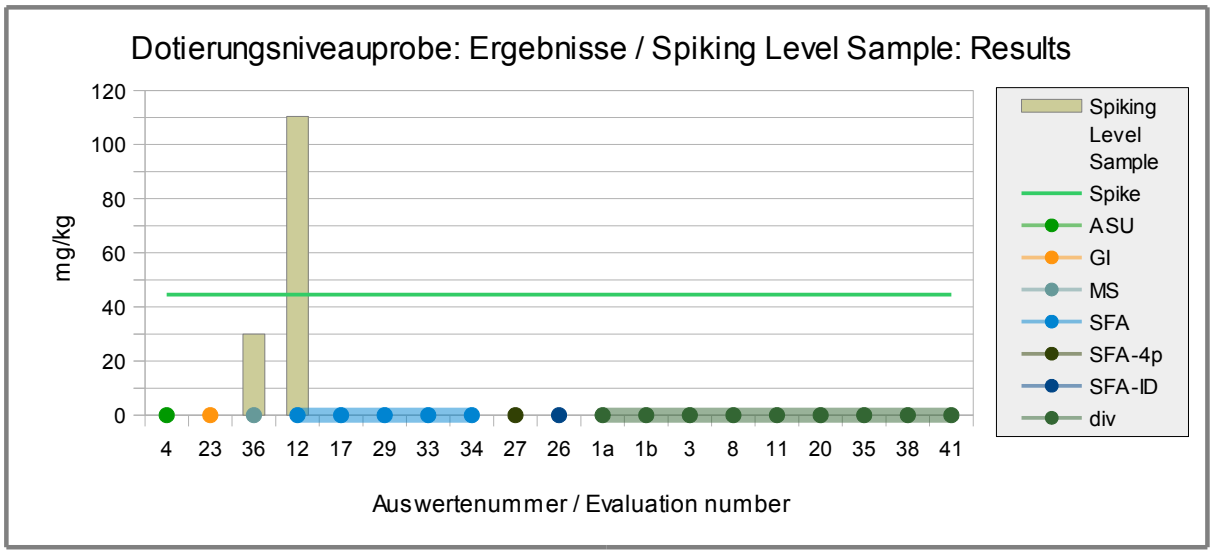


Abb./Fig. 14: PCR-Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4					ASU	
23					GI	
36	30,0	**67**	20,0	40	MS	
12	110	248	54,7	**111**	SFA	
17					SFA	
29					SFA	
33					SFA	
34					SFA	
27					SFA-4p	
26					SFA-ID	
1a					div	
1b					div	
3					div	
8					div	
11					div	
20					div	
35					div	
38					div	
41					div	

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

* 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 = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
Each of the participants optained for the spiking level sample or for the spiked food matrix sample A a recovery rate by PCR methods within the range of the AOAC-recommendation of 50-150%.

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		
14	positive	7,80	negative	<2	2/2 (100%)	AQ	
23	positive	19,3	negative	<0,86	2/2 (100%)	AQ	Result converted °
9	positive	4,40	negative	<2	2/2 (100%)	BC	
12	positive	2,77	negative	<2	2/2 (100%)	BC	
40	positive	8,90	negative	0	2/2 (100%)	BF	
4	positive	5,40	negative	<2,0	2/2 (100%)	EF	
10a	positive	19,7	negative	< 2,0	2/2 (100%)	EF	
21	positive	12,0	negative	<2,0	2/2 (100%)	EF	
39	positive	4,30	negative	<2,0	2/2 (100%)	EF	
7	positive	2,88	negative	<0,54	2/2 (100%)	ES	Result converted °
30	positive	27,5	negative	<1,1	2/2 (100%)	ES	Result converted °
22	positive	15,3	negative	<2	2/2 (100%)	IL	
24	positive	36,6	negative	<8,6	2/2 (100%)	IL	Result converted °
32	positive	8,60	negative	0	2/2 (100%)	IL	
2	positive	78,3	negative		2/2 (100%)	RS-F	
5	positive	35,0	negative	<2,5	2/2 (100%)	RS-F	
6	positive	77,0	negative	<2,5	2/2 (100%)	RS-F	
10b	positive	>20	negative	< 2,5	2/2 (100%)	RS-F	
13	positive	58,5	negative		2/2 (100%)	RS-F	
15	positive	78,8	negative		2/2 (100%)	RS-F	
18	positive	84,0	negative	<2,5	2/2 (100%)	RS-F	
19	positive	29,6	negative	0	2/2 (100%)	RS-F	
25	positive	140	negative	<2,5	2/2 (100%)	RS-F	
27	positive		negative		2/2 (100%)	RS-F	
28	positive	125	negative	<2,5	2/2 (100%)	RS-F	
31	positive	>20	negative	<2,5	2/2 (100%)	RS-F	
34	positive	>20	negative	< 2,5	2/2 (100%)	RS-F	
37	positive	18,3	negative	<2,5	2/2 (100%)	RS-F	
42	positive	343	negative		2/2 (100%)	RS-F	Result converted °
16	positive	130	positive	9,6	1/2 (50%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	30		1	
Number negative	0		29	
Percent positive	100		3	
Percent negative	0		97	
Consensus value	positive		negative	

Methods:

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

Comments:

The consensus values are in qualitative agreement with the spiking of

sample A. For sample B a positive result was obtained with the method VT (Veratox).

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Sesame	z'-Score Xpt_{12}	z'-Score $Xpt_{>50}$	z'-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]					
14	7,80	-0,90			AQ	
23	19,3	1,8			AQ	Result converted °
9	4,40	-1,7			BC	
12	2,77	-2,1			BC	
40	8,90	-0,63			BF	
4	5,40	-1,5			EF	
10a	19,7	1,9			EF	
21	12,0	0,10			EF	
39	4,30	-1,7			EF	
7	2,88	-2,1			ES	Result converted °
30	27,5	3,8			ES	Result converted °
22	15,3	0,89			IL	
24	36,6	6,0			IL	Result converted °
32	8,60	-0,71			IL	
2	78,3		-0,23	-0,07	RS-F	
5	35,0		-1,8	-1,6	RS-F	
6	77,0		-0,28	-0,11	RS-F	
10b	> 20				RS-F	
13	58,5		-0,93	-0,78	RS-F	
15	78,8		-0,21	-0,05	RS-F	
18	84,0		-0,03	0,14	RS-F	
19	29,6		-1,9	-1,8	RS-F	
25	140		1,9	2,2	RS-F	
27					RS-F	
28	125		1,4	1,6	RS-F	
31	>20				RS-F	
34	>20				RS-F	
37	18,3		-2,3	-2,2	RS-F	
42	343		9,1	9,5	RS-F	Result converted °
16	130		1,6		VT	

° calculation see p. 19

Methods:

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

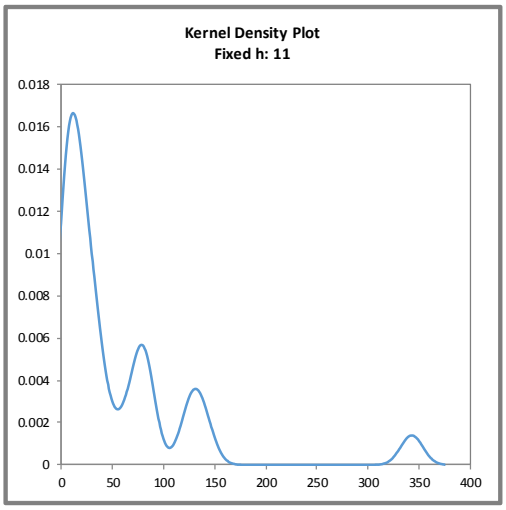


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a major peak at approx. 12 mg/kg with a nearly symmetrical distribution of results. All values above 50 mg/kg are due to results of the methods RS-F und VT and were therefore evaluated separately. There is a peak at approx. 80 mg/kg, a smaller peak at approx. 130 mg/kg and a small peak at 343 mg/kg, which is due to a single result.

Characteristics: Quantitative evaluation ELISA Sesame

Sample A

Statistic Data	**Methods Peak 12** *[mg/kg]*	**Methods >50** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{12}}$	$X_{pt_{>50}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	14	12	11
Number of outliers	0	1	1
Mean	12,5	99,8	97,1
Median	8,75	78,6	78,3
Robust Mean (X_{pt})	**11,6**	**84,9**	**80,1**
Robust standard deviation (S*)	**9,11**	**52,7**	**51,2**
Target range:			
Target standard deviation $\sigma_{pt'}$	**4,20**	**28,5**	**27,8**
lower limit of target range	**3,17**	**27,9**	**24,5**
upper limit of target range	**20,0**	**142**	**136**
Quotient S/$\sigma_{pt'}$*	*2,2*	*1,8*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*3,04*	*19,0*	*19,3*
Results in the target range	*10*	*10*	*8*
Percent in the target range	*71*	*83*	*73*

Method:
Peak 12 = AgraQuant, BioCheck, BioFront Technologies, Eurofins Technologies, ELISA Systems, Immunolab
>50 = Ridascreen® Fast, Veratox
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution of results with a major peak and three secondary peaks. Therefore no joint evaluation of all methods was carried out, but an evaluation of the methods that are assigned to the major peak ("peak 12"), and an evaluation of the methods that gave the results above 50 mg/kg ("methods >50") (Assignment see above below the table).
The distribution of the results of peak 12, the methods >50 as well as method RS-F showed an increased variability of results, with a quotient S/σpt above 2,0 each. Therefore the evaluations were done by z'-score considering the standard uncertainty. The quotients S*/$\sigma_{pt'}$ were then at 1,8-2,2.*
The robust standard deviations are increased in comparison to established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods.
The robust means of the evaluations were 37%, 274% and 259% of the spiking level of sesame to sample A and thus out of the range of the recom-

mendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA of Sesame" p.53).

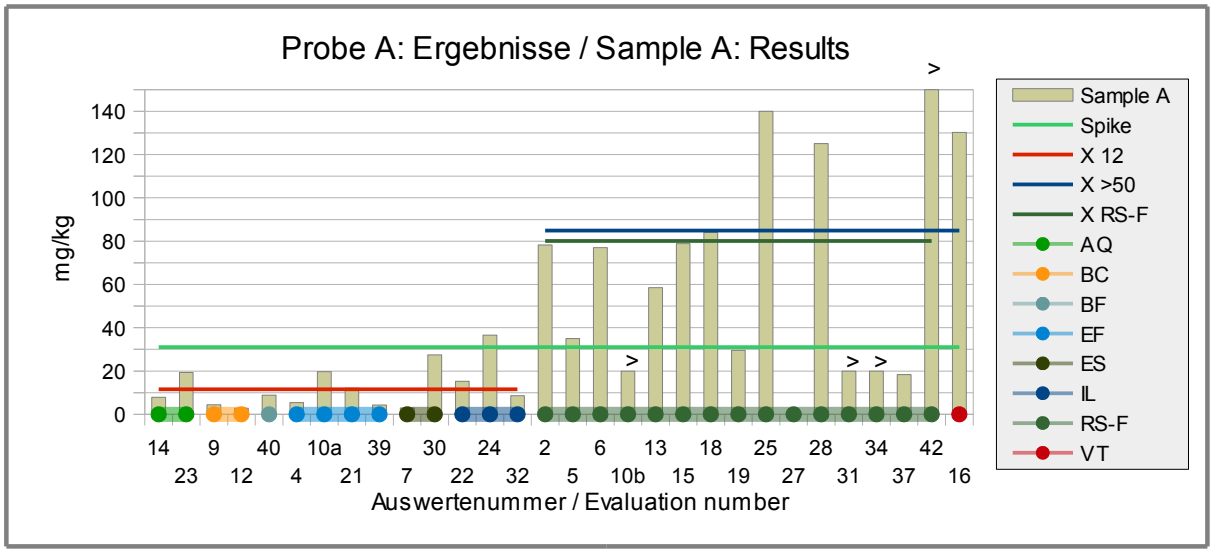


Abb./Fig. 16: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 12"
blue line = Assigned value robust mean all results of "methods >50"
darkgreen line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

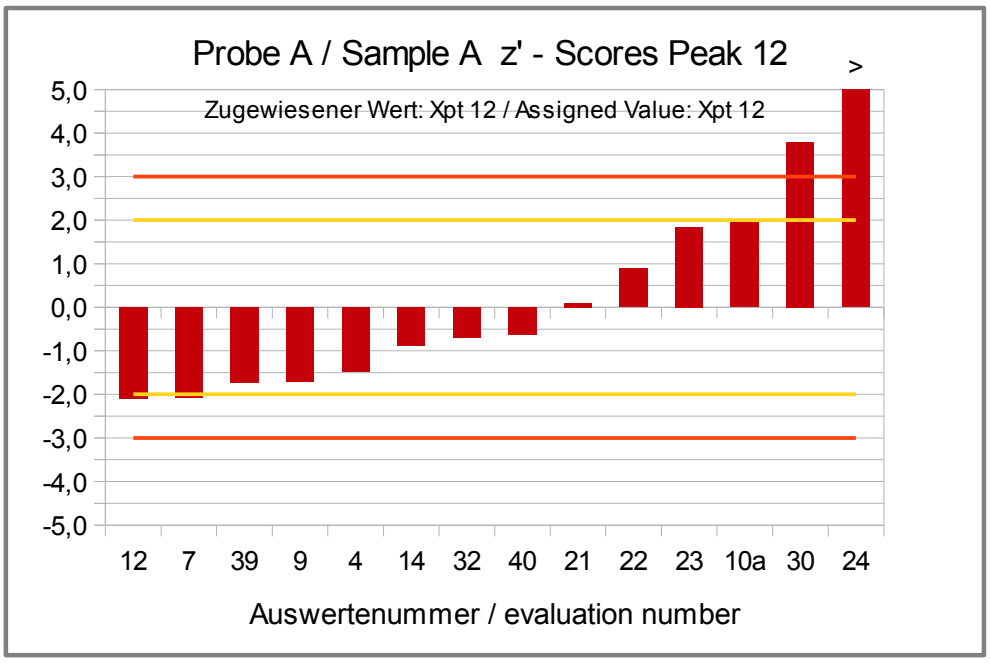


Abb./Fig. 17:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 12

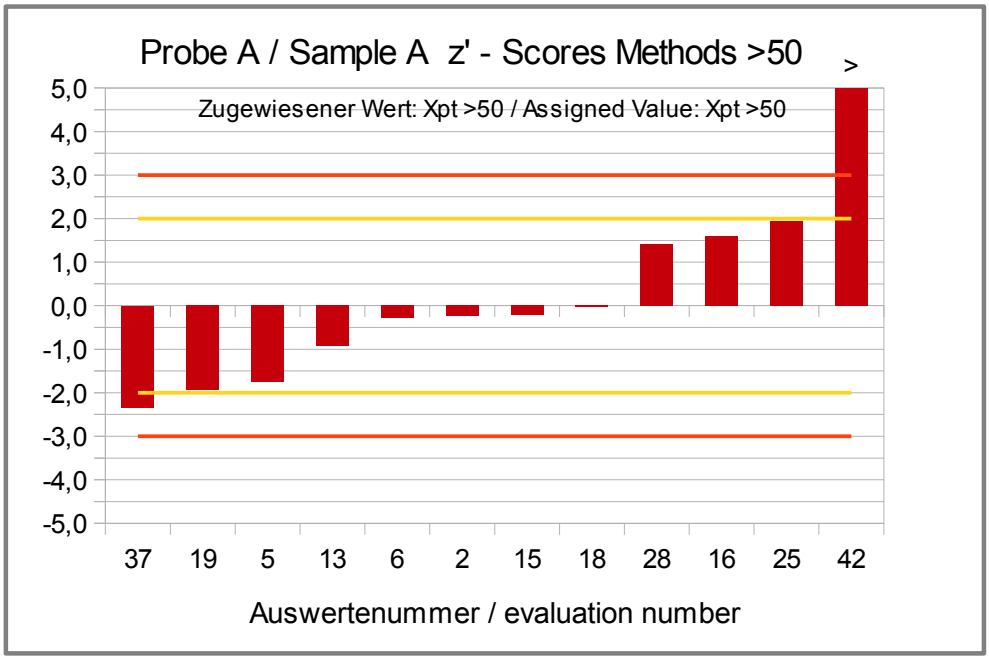


Abb./Fig. 18:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of methods >50

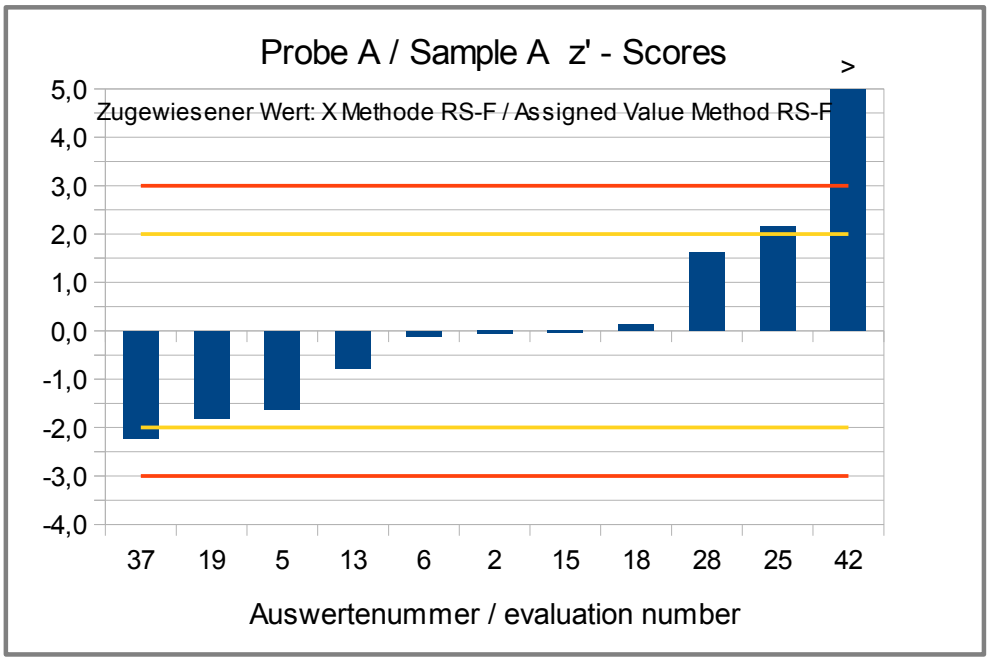


Abb./Fig. 19:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative Valuation of results: Spiking level sample

Evaluation number	Sesame	z'-Score Xpt_{23}	z'-Score Xpt_{85}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]					
14	16,9	-1,2			AQ	
23	104	8,8			AQ	Result converted °
9	14,1	-1,5			BC	
12	17,1	-1,2			BC	
40	41,2	1,6			BF	
4	16,0	-1,3			EF	
10a	22,0	-0,60			EF	
21	20,5	-0,78			EF	
39	15,0	-1,4			EF	
7					ES	Result converted °
30	42,1	1,7			ES	Result converted °
22	27,0	-0,03			IL	
24	86,4	6,8			IL	Result converted °
32	21,0	-0,72			IL	
2	85,4		-0,11	0,19	RS-F	
5	80,0		-0,31	-0,08	RS-F	
6	72,0		-0,62	-0,47	RS-F	
10b	> 20,0				RS-F	
13	58,8		-1,1	-1,1	RS-F	
15	65,1		-0,88	-0,81	RS-F	
18	110		0,82	1,4	RS-F	
19	28,3		-2,3	-2,6	RS-F	
25	92,0		0,14	0,51	RS-F	
27					RS-F	
28	104		0,58	1,1	RS-F	
31	>20				RS-F	
34	>20				RS-F	
37	67,7		-0,78	-0,68	RS-F	
42	374		11	14	RS-F	Result converted °
16	222		5,1		VT	

° calculation see p. 19

Methods:

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

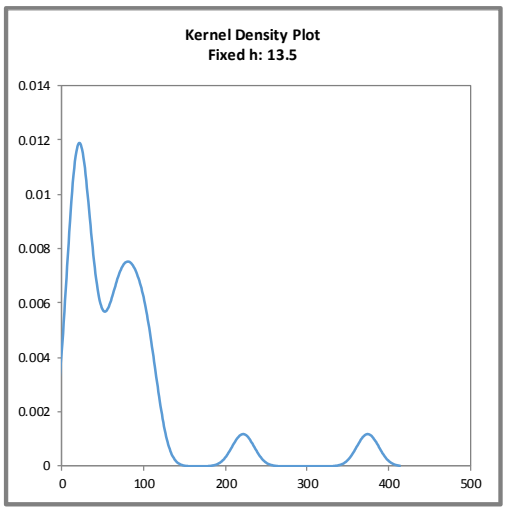


Abb. / Fig. 20:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a major peak at approx. 23 mg/kg with a nearly symmetrical distribution of results. Furthermore there are a second peak at approx. 85 mg/kg and two smaller side peaks at approx. 222 mg/kg and 374 mg/kg, which are due to single results. The higher values are based on the results of the methods RS-F and VT with two exceptions and were therefore evaluated separately.

Characteristics: Quantitative evaluation ELISA Sesame

Spiking Level Sample

Statistic Data	**Methods Peak 23** *[mg/kg]*	**Methods Peak 85** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{23}}$	$X_{pt_{85}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	13	12	11
Number of outliers	–	–	–
Mean	34,1	113	103
Median	21,0	82,7	80,0
Robust Mean (X_{pt})	**27,3**	**88,3**	**81,6**
Robust standard deviation (S*)	**15,7**	**40,0**	**27,6**
Target range:			
Target standard deviation $\sigma_{pt'}$ or σ_{pt}	**8,72**	**26,4**	**20,4**
lower limit of target range	**9,83**	**35,5**	**40,8**
upper limit of target range	**44,7**	**141**	**122**
Quotient S/$\sigma_{pt'}$ or S*/σ_{pt}*	*1,8*	*1,5*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*5,43*	*14,4*	*10,4*
Results in the target range	*11*	*9*	*9*
Percent in the target range	*85*	*75*	*82*

Method:
Peak 23 = AgraQuant, BioCheck, BioFront Technologies, Eurofins Technologies, ELISA Systems, Immunolab
Peak 85 = Ridascreen® Fast, Veratox
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution of results with a major peak and a secondary peak (as well as two additional small peaks of single results). Therefore no joint evaluation of all methods was carried out, but two evaluations separated by methods, which were assigned to the major peak (peak 23, with two higher results) or assigned to the secondary peak (peak 85)(Assignment see above under the table).
The distributions of the results of peak 23 and peak 85 showed an increased variability of results, with quotients S/σpt above 2,0 each. Therefore the evaluations were done by z'-score considering the standard uncertainty. The quotients S*/$\sigma_{pt'}$ were then below 2,0. The evaluation of method RS-F showed a normal variability of results, with a quotient S*/σpt of 1,4.*
The robust standard deviations for peak 23 and 85 are increased while the robust standard deviation of method RS-F is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods.

The robust means of the evaluations were 98%, 316% and 293% of the spiking level of sesame to the spiking level sample within or above the

range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA for Sesame" p.53).

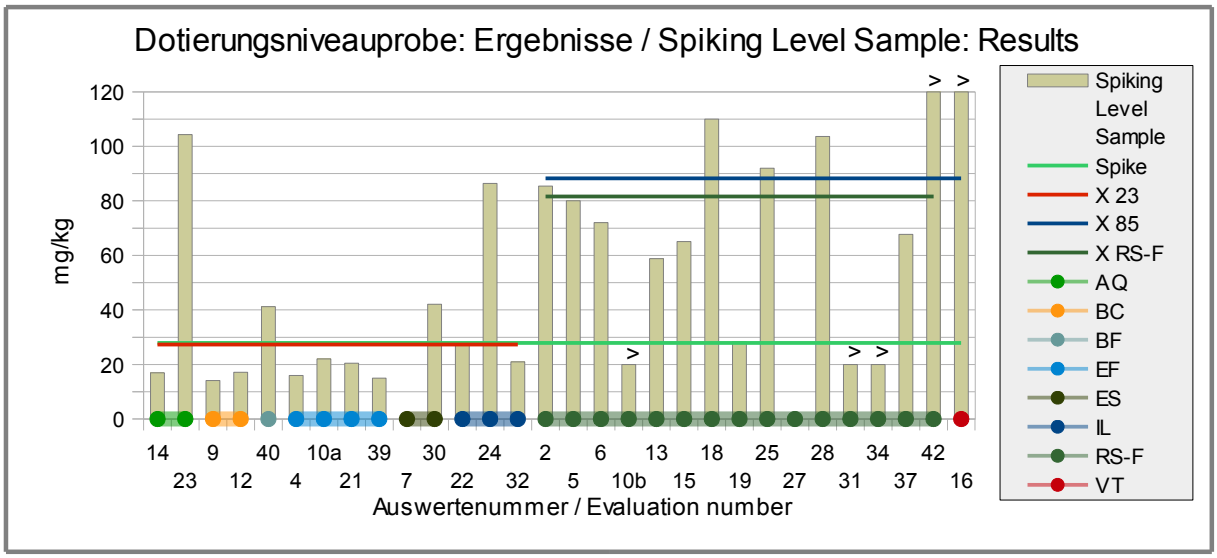


Abb./Fig. 21: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 23"
blue line = Assigned value robust mean all results of "peak 85"
darkgreen line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

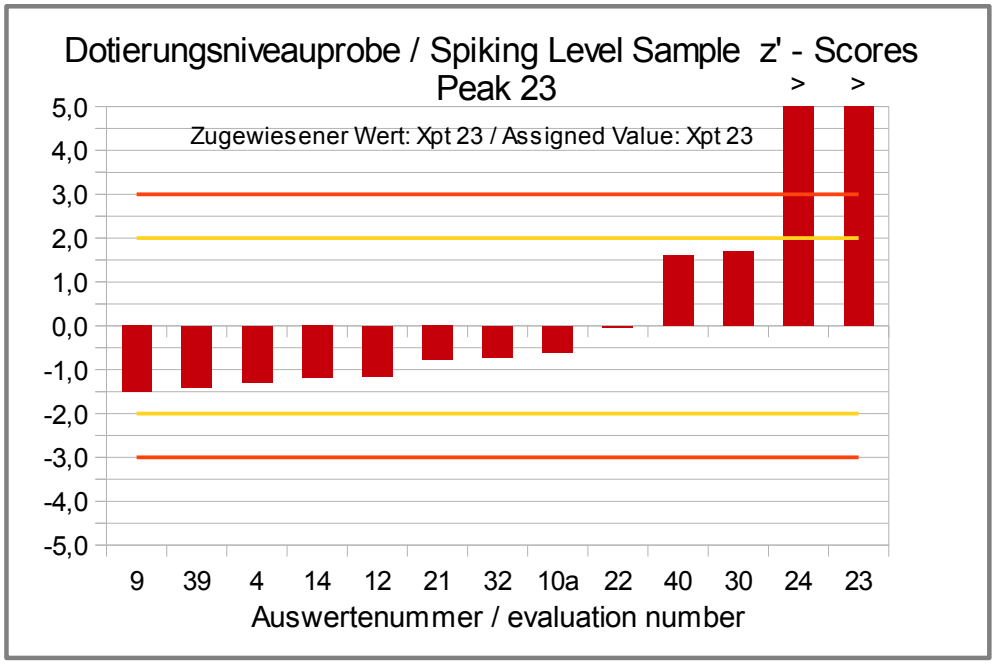


Abb./Fig. 22:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 23

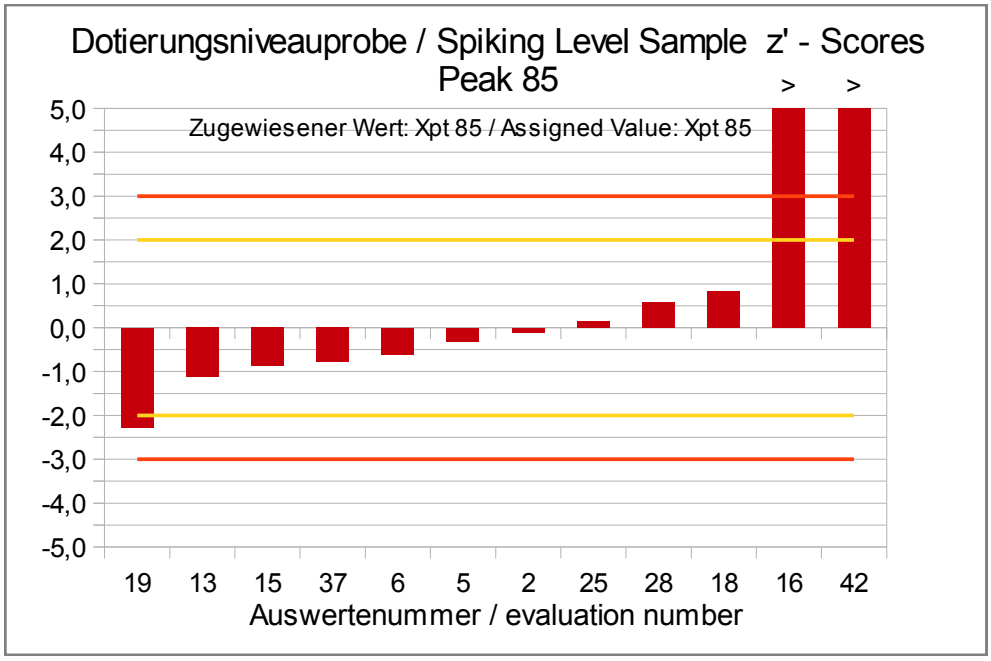


Abb./Fig. 23:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 85

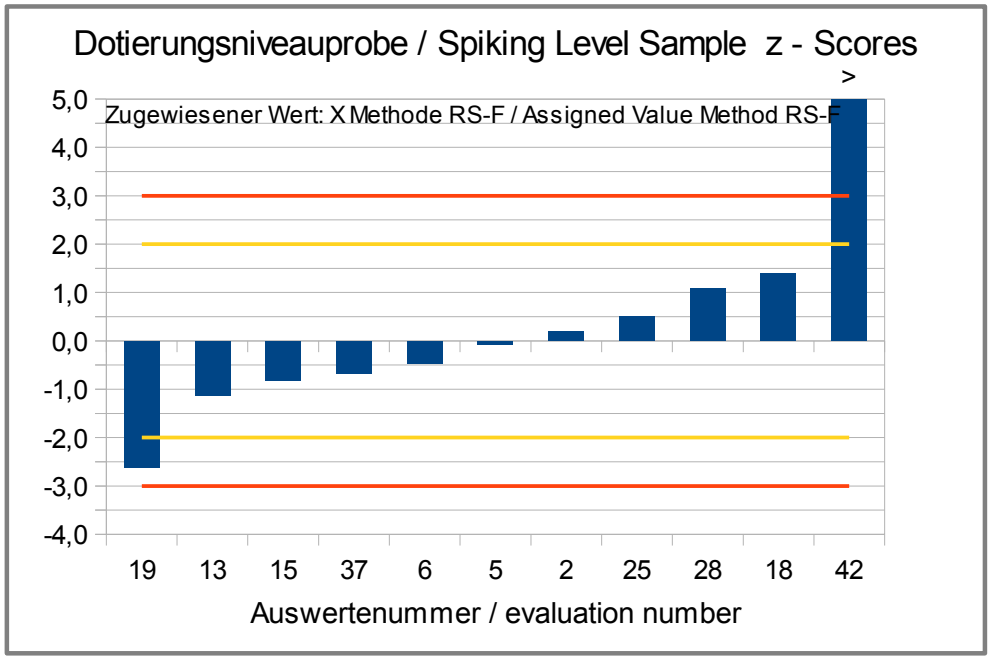


Abb./Fig. 24:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
14	16,9	**61**	7,80	25	AQ	
23	104	374	19,3	**62**	AQ	Result converted °
9	14,1	**51**	4,40	14	BC	
12	17,1	**61**	2,77	8,9	BC	
40	41,2	**148**	8,90	29	BF	
4	16,0	**57**	5,40	17	EF	
10a	22,0	**79**	19,7	**64**	EF	
21	20,5	**73**	12,0	39	EF	
39	15,0	**54**	4,30	14	EF	
7			2,88	9,3	ES	Result converted °
30	42,1	151	27,5	**89**	ES	Result converted °
22	27,0	**97**	15,3	49	IL	
24	86,4	310	36,6	**118**	IL	Result converted °
32	21,0	**75**	8,60	28	IL	
2	85,4	306	78,3	253	RS-F	
5	80,0	287	35,0	**113**	RS-F	
6	72,0	258	77,0	248	RS-F	
10b	> 20		> 20		RS-F	
13	58,8	211	58,5	189	RS-F	
15	65,1	233	78,8	254	RS-F	
18	110	394	84,0	271	RS-F	
19	28,3	**101**	29,6	**95**	RS-F	
25	92,0	330	140	452	RS-F	
27					RS-F	
28	104	371	125	403	RS-F	
31	> 20		>20		RS-F	
34	> 20		>20		RS-F	
37	67,7	243	18,3	**59**	RS-F	
42	374	1340	343	1108	RS-F	Result converted °
16	222	794	130	420	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**7**
Percent in RA	**44**	Percent in RA	**27**

* 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 = SensiSpec ELISA Kit, Eurofins
ES = ELISA-Systems
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

44% (11) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150% with the spiking level sample. For the spiked food matrix sample A 27% (7) of the recovery rates were within 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		
4	positive		negative		2/2 (100%)	ASU	
13	positive		negative		2/2 (100%)	ASU	
23	positive		negative		2/2 (100%)	GI	
11	positive		negative		2/2 (100%)	MS	
36	positive	20,0	negative		2/2 (100%)	MS	
12	positive	2,85	negative	<1	2/2 (100%)	SFA	
17	positive		negative		2/2 (100%)	SFA	
34	positive		negative		2/2 (100%)	SFA	
35	positive		negative		2/2 (100%)	SFA-ID	
1	positive		negative		2/2 (100%)	div	
3	positive		negative	8	2/2 (100%)	div	
8	positive		negative		2/2 (100%)	div	
20	positive		negative		2/2 (100%)	div	
29	positive		negative		2/2 (100%)	div	
41	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	15		0	
Number negative	0		15	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

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 = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative Valuation PCR: Sample A

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

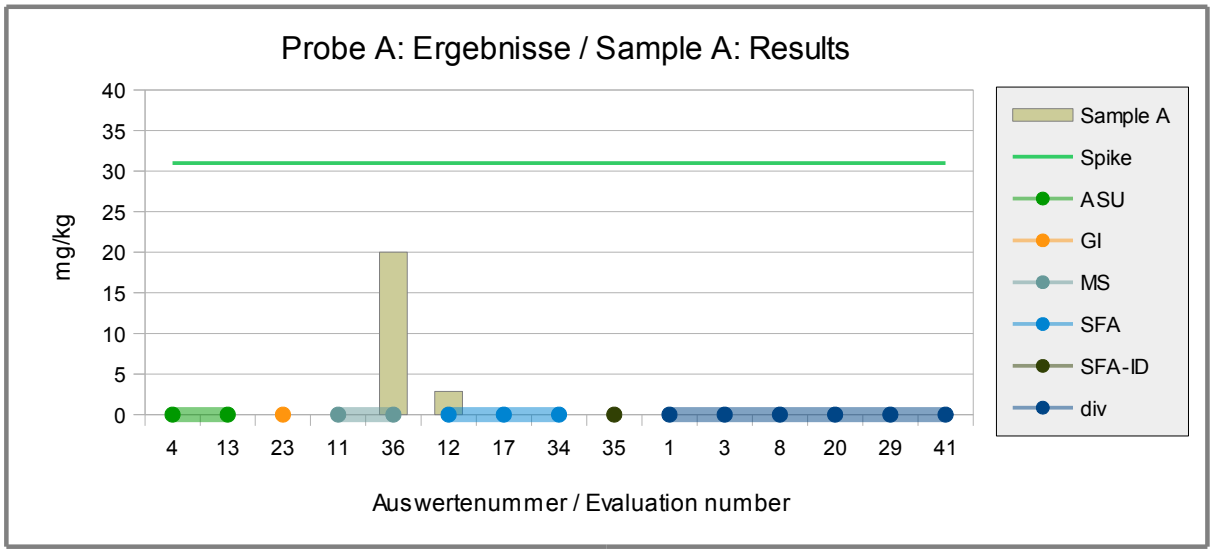


Abb./Fig. 25: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

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

Evaluation number	Sesame	Sesame	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive			ASU	
13	positive			ASU	
23	positive			GI	
11	positive			MS	
36	positive	60,0		MS	
12	positive	6,49		SFA	
17	positive			SFA	
34	positive			SFA	
35	positive			SFA-ID	
1	positive			div	
3				div	
8	positive			div	
20	positive			div	
29	positive			div	
41	positive			div	

	Sample B	
Number positive	14	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:
Methoden:
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 = keine genaue Angabe / andere Methode
div = not indicated / other method

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

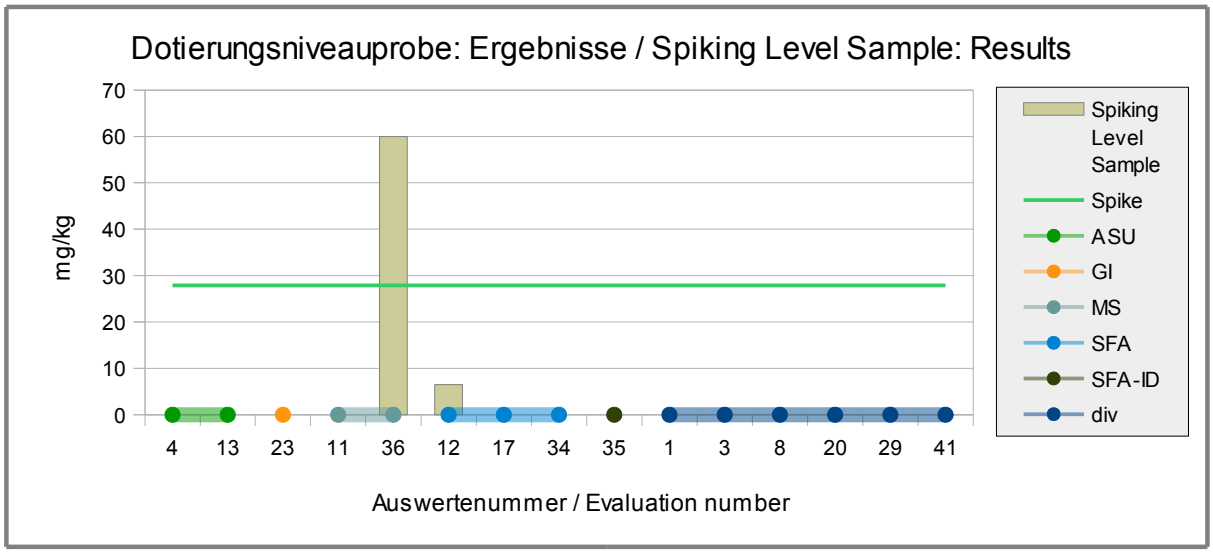


Abb./Fig. 26: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4					ASU	
13					ASU	
23					GI	
11					MS	
36	60,0	215	20,0	**65**	MS	
12	6,49	23	2,85	9,2	SFA	
17					SFA	
34					SFA	
35					SFA-ID	
1					div	
3					div	
8					div	
20					div	
29					div	
41					div	

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

* Recovery rate 100% relative size: 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 = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
None of the two participants obtained a recovery rate by PCR methods within the range of the AOAC-recommendation of 50-150% with the spiking level sample. For the spiked food matrix sample A one of the recovery rates were within the range of acceptance.

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 no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	14	11.06.19	positive	29	negative	<2	positive	122,6		2	48,5	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	21	09.07.19	positive	33,8	negative	<2.0	positive	139,3	0,2	2		Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	23	11/July	positive	34,8	negative	<1	positive	101,4	1	2	15	Mustardprotein	AgraQuant ELISA Mustard COKAL2148, RomerLabs
BC	9	17.07.19	-	25,4	-	<2	-	96,5	2	2	50	Mustard	BioCheck ELISA Mustard-Check
BF	40	23/7	positive	37	negative	0	positive	49	0,13	1		Mustard	MonoTrace Mustard ELISA kit, BioFront Technologies
EF	10a		-	62,94	-	< 2,0	-	> 60		2		Mustard	SensiSpec ELISA Sesame, Eurofins
IL	22		positive	63,4	negative	<2	positive	99,2	2	2		Mustard	Immunolab Mustard ELISA
IL	32		positive	33,6	negative	<0,04	positive	106,25				Mustard	Immunolab Mustard ELISA
RS-F	10b	19.07.19	-	47,8	-	< 0,5	-	> 13,5		0,5		Mustard	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	12	13.06.19	positive	83,11	negative	<0.5	positive	82,25	0,5	0,5	28,72	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	13	18.06.19	positive	64,2	negative		positive	72,1	0,5	0,5	42	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	17	15.07.19	-	35,6	-	<0,5	-	19,7	0,1	0,5	40	Mustardprotein	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	18	08.07.2019	positive	69	negative	<0.5	positive	65	DLA2019	0.5	39,4	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	19	17.07.19	positive	15,8	negative	0	positive	13,2	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	27		positive		negative		positive					Please select!	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	28	01.07.19	pos	69,4	neg	<0.5	pos	64,4	0,5	0,5	31	Mustard Seed	R-Biopharm FAST Mustard
RS-F	31	19.06.19	positive	>13,5	negative	<0,5	positive	>13,5		0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
VT	2	13.06.19	positive	64,5	negative		positive	102		2,5		Mustard	Veratox Mustard, Neogen
VT	4	25.06.19	positive	56	negative	<2,5	positive	105	1,5	2,5		Mustard	Veratox Mustard, Neogen
VT	7	26.06.19	Pos	64,1	Neg	<1.0	not tested		1	2,5		mustard	Veratox Mustard, Neogen
VT	15	16.07.19	positive	42,1	negative		positive	79,7		2,5		Mustard	Veratox Mustard, Neogen
VT	16	09.07.19	positive	56,2	negative	<2.5	positive	52,9		2,5		Mustard	Veratox Mustard, Neogen
VT	30	19.06.19	positive	52	negative	<2.5	positive	63	2,5	2,5	23	Mustard	Veratox Mustard, Neogen
VT	39	26.06.19	positive	50,5	negative	<2,5	positive	66	2,5	2,5	50	Mustard	Veratox Mustard, Neogen
VT	42	17.07.19	positive	23,5	negative		positive	52,3	1	2,5	25	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 no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	14			Yes	
AQ	21			yes	
AQ	23			yes	
BC	9		0.5g sample 10ml extraction buffer/15min/60C	Yes	
BF	40	Monoclonal-based assay	1:20 extraction ratio/10 minutes/60C	no	
EF	10a			yes	
IL	22	yellow mustard	As Per Kit Instructions		Crossreactivity with brown (59%) and black mustard (50%)
IL	32				
RS-F	10b			yes	
RS-F	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	13			yes	
RS-F	17		extraction with buffer (milk if spice presence)/10'/60°C	yes	
RS-F	18	Mustard protein (not specified by provider)	As per kit instructions	no	
RS-F	19		Extraction: with Allergen extraction buffer, 10 min., 60 °C	no	
RS-F	27			No	
RS-F	28	Unknown	1g in 20ml buffer, 10-10-10 ELISA incubations, 1:20 sample dilution to quantify	Yes	
RS-F	31			no	
VT	2		as per kit insert	yes	
VT	4	mustard proteins from white, black and brown mustard	according to manufacturer instructions	yes	
VT	7		Extraction: 60C pre-heated TRIS extraction buffer/ samples extracted in shaking waterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	Yes	
VT	15				
VT	16				
VT	30	Poly/Mono	Tris EDTA Solution / 15 min / 60 C	yes	Single Result
VT	39			yes	Performed according to Kit-instruction
VT	42		Veratox mustard extr. Buffer / 15 min. / 60°C	yes	

5.1.2 ELISA: Sesame

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	14	11.06.19	positive	7,8	negative	<2	positive	16,9		2	66,7	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
AQ	23	10/July	positive	4,5	negative	<0,2	positive	24,3	0,2	2	15	Sesameprotein	AgraQuant ELISA Sesame COKAL1948, RomerLabs
BC	9	25.06.19	-	4,4	-	<2	-	14,1	2	2	50	Sesame	BioCheck ELISA Sesame-Check
BC	12	19.06.19	positive	2,77	negative	<2	positive	17,13	2	2	28,44	Sesame	BioCheck ELISA Sesame-Check
BF	40	23/7	positive	8,9	negative	0	positive	41,2	0,22	1		Sesame	MonoTrace Sesame ELISA kit, BioFront Technologies
EF	4	20.06.19	positive	5,4	negative	<2,0	positive	16	1,5	2		Sesame	SensiSpec ELISA Sesame, Eurofins
EF	21	05.07.19	positive	12	negative	<2.0	positive	20,5	0,2	2		Sesame	SensiSpec ELISA Sesame, Eurofins
EF	10a		-	19,73	-	< 2,0	-	21,99		2		Sesame	SensiSpec ELISA Sesame, Eurofins
EF	39	02.07.19	positive	4,3	negative	<2,0	positive	15	2	2	50	Sesame	SensiSpec ELISA Sesame, Eurofins
ES	7	21.06.19	Pos	0,67	Neg	<0.125	not tested		0,125	0,25		sesame protein	Sesame, ELISA Systems
ES	30	21.06.19	positive	6,4	negative	<0.25	positive	9,8	0,25	0,25	29	Sesameprotein	ELISA Systems Sesame ESSESE-48
IL	22		positive	15,3	negative	<2	positive	27	2	2		Sesame	Immunolab Sesame ELISA
IL	24	20.06.19	-	8,52	-	<2	-	20,14	0,2	2		Sesameprotein	Immunolab Sesame ELISA
IL	32		positive	8,6	negative	0	positive	21				Sesame	Immunolab Sesame ELISA
RS-F	2	14.06.19	positive	78,3	negative		positive	85,4		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	5	17.07.19	positive	35	negative	<2,5	positive	80	0,14	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	6	12.07.19	positive	77	negative	<2.5	positive	72	0,2	2,5	19	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	10b	19.07.19	-	> 20	-	< 2,5	-	> 20,0		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	13	26.07.19	positive	58,5	negative		positive	58,8	2,5	2,5	20	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	15	11.07.19	positive	78,8	negative		positive	65,1		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	18	08.07.2019	positive	84	negative	<2.5	positive	110	2,5	2,5	38,6	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	19	17.07.19	positive	29,6	negative	0	positive	28,3	0,14	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	25	11.07.19	positive	140	negative	<2.5	positive	92		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	27		positive		negative		positive					Please select!	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	28	01.07.19	pos	125	neg	<2.5	pos	103,6	2,5	2,5	27	Sesame Seed	R-Biopharm FAST Sesame
RS-F	31	19.06.19	positive	>20	negative	<2,5	positive	>20		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	34	17.06	positive	>20	negative	< 2,5	positive	>20	0,14	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	37	18.06.19	positive	18,3	negative	<2,5	positive	67,7	0,14	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	42	17.07.19	positive	80	negative		positive	87,1	0,24	2,5	25	Sesameprotein	Ridascreen® FAST Sesame R7202, R-Biopharm
VT	16	03.07.19	positive	130,2	positive	9,6	positive	221,5		2,5		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 no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	14			Yes	
AQ	23			yes	
BC	9		0.5g sample 10ml extraction buffer/15min/60C	Yes	
BC	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
BF	40	Monoclonal-based assay	1:20 extraction ratio/10 minutes/60C	no	
EF	4	Sesameproteins	according to manufacturer instructions	yes	
EF	21			yes	
EF	10a			yes	
EF	39			yes	Performed according to Kit-instruction
ES	7		Extraction: Room temperature PBS extraction buffer (pH check) and samples extracted in shaking waterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	Yes	
ES	30	Polyclonal/ Monoclonal	Extraction solution concentrate / 15 mins / 60C	yes	Single Result
IL	22		As Per Kit Instructions		
IL	24		Extraction buffer/15min/60 C	yes	
IL	32				
RS-F	2		as per kit insert, extraction with 5% milk powder	yes	low recovery in sample B (46%)
RS-F	5			no	
RS-F	6	Sesame protein - not specified by manufacturer	Samples extracted using SMP-AEP, 60C, with shaking for 10 minutes, then cooled and centrifuged at 2500g	Yes	A spike receovery test on sample B indicated that there was interference from the matrix used for samples A and B
RS-F	10b			yes	
RS-F	13			yes	
RS-F	15				
RS-F	18	Sesame protein (not specified by provider)	As per kit instructions	no	
RS-F	19		Extraction: with Allergen extraction buffer containing milk powder, 10 min., 60 °C	no	
RS-F	25			No	
RS-F	27				
RS-F	28	Unknown	1g in 20ml buffer, 10-10-10 ELISA incubations, 1:20 sample dilution to quantify	Yes	
RS-F	31			no	
RS-F	34			yes	
RS-F	37		extraction according to kit-insert	no	Method not verified/validated at our laboratory yet, so M.U is not calculated.
RS-F	42		Allergen extr. Buffer / 10 min. / 60°C	yes	
VT	16				

5.1.3 PCR: Celery

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
ASU	4	01.07.19	positive	Traces at LOD	negative		positive		50			Celery-DNA	ASU §64 Methode/method
ASU	13	27.06.19	positive		negative		positive					Celery-DNA	ASU §64 Methode/method
ASU	26		positive		negative		positive		10	20		Celery-DNA	ASU §64 Methode/method
ASU	35	13.06.19	positive		negative		positive		5	10		Celery-DNA	ASU §64 Methode/method
FP	23a	8/July	positive	0,86	negative		positive	0,42	0,01	0,08	30	Celery-DNA	foodproof Detection Kit, BIOTECON Diagnostics
GI	23b	9/July	positive		negative		positive		5 gene copies			Celery-DNA	GEN-IAL First Allergen
IM	33	20.06.19	positive		negative		positive		0,4			Celery	other: Imegen Celery ID kit
MS	11		positive		negative		positive		0,01% DNA			please select!	Microsynth
MS	36	20.06.19	positive	70	negative		positive	100	10	100	250	Food	Microsynth
SFA	10	19.07.19	positive	7,61	positive	1	positive	3,08		1		Celery	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	21.06.19	positive	28,74	negative	<1	positive	35,16	1	1	25,06	Celery	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	15.07.19	positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18	08.07.19	positive		positive		positive		1	1		Celery	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	22		positive		negative		positive					Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	29	26.06.19	pos		neg		pos		0,5			n/a	Surefood Allergen Celery, Congen
SFA	34	19.06	positive		positive		positive		0,4			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	27		positive		negative		positive					Please select!	Sure Food Allergen 4plex, R-Biopharm / Congen
div	1		positive		negative		positive		5	nd		Please select!	CEN/TS 15634-2
div	3	13.06.19	pos		neg	8	-						in-house method
div	8	19.07.19	negative		negative		positive		100			Please select!	other: Internal methods
div	14	17.07.19	positive		negative		negative		10			Celery-DNA	In House
div	20	15.07.19	positive		negative		positive		10			Please select!	in house method
div	28	01.07.19	pos		neg		pos		1	1	N/A	DNA	In-House method
div	38	19/June	positive		negative		positive					Please select!	other: ISO CEN/TS 15634-2:2012
div	41		positive		negative		positive					Please select!	in house method

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation PCR Celery:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4		CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Realtime PCR / 45 cycles	yes	§64 LFGB L 08.00-56:2014-08
ASU	13	Mannitol dehydrogenase	Macherey & Nagel NucleoSpin Food Kit	yes	From sample A, only very little DNA could be extracted. The results for celery are not clear.
ASU	26	Mannitol-Dehydrogenase	CTAB-Extraction with Magnetic Bead-Clean up	yes	
ASU	35	Mannitol-Dehydrogenase	CTAB precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
FP	23a			no	
GI	23b			yes	
IM	33		CTAB/real time PCR/50cycles	NO	
MS	11	AF067082	Macherey Nagel Nucleo Spin Food with optimizations: increased weight, chloroform step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermal profile; inhibition control	yes	
MS	36		Wizard	yes	Spiking below LOQ of routine
SFA	10			yes	
SFA	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA	17		extraction with kit Congen Sure Food PREP Advanced / real time PCR / 45 cycles	yes	
SFA	18	Celery DNA (not specified by provider)	As per kit instructions	no	
SFA	22		as Per Kit Instructions		
SFA	29		CTAB-extraction followed by kit based DNA-pruification	yes	
SFA	34			yes	
SFA-4p	27			yes	
div	1	Manitol déshydrogenase	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	3		Limit of detection given as µg of DNA per kg of sample	no	
div	8	mitochondrial genes	MN extraction Kit + Real time PCR	Yes	Trace amount detected below LOD by NGS
div	14		Gel Electrophoresis	Yes	
div	20		CTAB Extraction + real time PCR	no	
div	28	MtD	Tris extraction with column clean-up, Real-Time PCR.	Yes	Qualitative only
div	38	mannitol dehydrogenase gene	CTAB/Proteinase K/RealTime PCR/45 cycles	no	
div	41		In-house method		

5.1.4 PCR: Mustard

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
ASU	4	01.07.19	positive		negative		positive		10			Mustard-DNA	ASU §64 Methode/method
GI	23	9/July	positive		negative		positive		5 gene copies			Mustard-DNA	GEN-IAL First Duplex Mustard PCR kit
MS	36	20.06.19	positive	20	negative		positive	30	10	100	250	Food	Microsynth
SFA	12	19.06.19	positive	54,73	negative	<1	positive	110,38	1	1	30,87	Mustard	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	15.07.19	positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	29	26.06.19	pos		neg		pos		0,5			n/a	Surefood Allergen Mustard, Congen
SFA	33	20.06.19	positive		negative		positive		0,4			Mustard	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	34	19.06	positive		negative		positive		0,4			Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	27		positive		negative		positive					Please select!	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	26		positive		negative		positive		10	20		Mustard-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1a		positive		negative		positive		5	nd		Please select!	Fuchs M., Cichna-Markl M., Hochegger, R – Development and validation of a real-time PCR method for the detection of white mustard (Sinapis alba) in foods. J. Agric. Food Chemis. 2010, 58, 11193-11200.
div	1b		negative		negative		positive (traces)		nd	nd		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 brown mustard (Brassica juncea) - Food Chemistry 138 (2013) 348-355
div	3	12.06.19	pos		neg	8	-						in-house method
div	8	19.07.19	positive		negative		positive		100			Please select!	other: Internal methods
div	11		positive		negative		positive		0,01% DNA			Please select!	in house method
div	20	15.07.19	positive		negative		positive		10			Please select!	in house method
div	35	21.06.19	positive		negative		positive		0,4	1		Mustard-DNA	Mustorp et al. 2008 Eur Food Res Technol. 226: 771-778
div	38	19/June	positive		negative		positive					Please select!	other: ISO CEN/TS 15634-5:2016
div	41		positive		negative		positive					Please select!	in house method

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation PCR Mustard:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4		CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Realtime PCR / 45 cycles	yes	§64 LFGB L 08.00-65:2017-10
GI	23			yes	
MS	36		Wizard	yes	Spiking below LOQ of routine
SFA	12	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA	17		extraction with kit Congen Sure Food PREP Advanced / real time PCR / 45 cycles	yes	
SFA	29		CTAB-extraction followed by kit based DNA-pruification	yes	
SFA	33		CTAB/real time PCR/45cycles	NO	
SFA	34			yes	
SFA-4p	27			yes	
SFA-ID	26	Mustard major allergen	CTAB-Extraction with Magnetic Bead-Clean up	yes	
div	1a	MADS-D	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	1b	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	3		Limit of detection given as µg of DNA per kg of sample	no	
div	8	mitochondrial genes	MN extraction Kit + Real time PCR	No	
div	11	MADS D/ Brassica juncea+nigra	Macherey Nagel Nucleo Spin Food with optimizations: increased weight, chloroform step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermal profile; inhibition control	yes	Sinapis alba: detectable; Brassica juncea/nigra: both in sample A and spiking level sample traces below NWG
div	20		CTAB Extraction + real time PCR	no	
div	35	Major allergen sin a1	CTAB precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
div	38	MADS-D protein gene	CTAB/Proteinase K/RealTime PCR/45 cycles	no	
div	41		in house method		

5.1.5 PCR: Sesame

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
ASU	4	01.07.19	positive		negative		positive		50			Sesame-DNA	ASU §64 Methode/method
ASU	13	21.06.19	positive		negative		positive					Sesame-DNA	ASU §64 Methode/method
GI	23	10/July	positive		negative		positive		5 gene copies			Sesame-DNA	GEN-IAL First Allergen
MS	11		positive		negative		positive		0,005 % DNA			Please select!	Microsynth
MS	36	20.06.19	positive	20	negative		positive	60	10	100	250	Food	Microsynth
SFA	12	17.07.19	positive	2,85	negative	<1	positive	6,49	1	1	30,52	Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	15.07.19	positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	34	19.06	positive		negative		positive		0,4			Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	35	13.06.19	positive		negative		positive		0,4	1		Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		positive		negative		positive		5	nd		Please select!	Waiblinger H-U - Ring trial validation of single and multiplex 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	3	13.06.19	pos		neg	8	-						in-house method
div	8	19.07.19	positive		negative		positive		10			Please select!	other: Internal methods
div	20	15.07.19	positive		negative		positive		10			Please select!	in house method
div	29	26.06.19	pos		neg		pos		10			n/a	inhouse Method
div	41		positive		negative		positive					Please select!	in house method

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation PCR Sesame:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4		CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Realtime PCR / 45 cycles	yes	§64 LFGB L 08.00-19:2014-08
ASU	13	2S Albumin Gen		yes	
GI	23			yes	
MS	11	U97700	Macherey Nagel Nucleo Spin Food with optimizations:increased weight, chloroform step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermal profile; inhibition control	yes	
MS	36		Wizard	yes	Spiking below LOQ of routine
SFA	12	As Per Kit Instructions	As Per Kit Instructions	No	Sesame not given as an option in drop down menu for 'Result given as'
SFA	17		extraction with kit Congen Sure Food PREP Advanced / real time PCR / 45 cycles	yes	
SFA	34			yes	
SFA-ID	35	Sesame	CTAB precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
div	1	Albumine 2S	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	3		Limit of detection given as µg of DNA per kg of sample	no	
div	8	mitochondrial genes	MN extraction Kit + Real time PCR	Yes	
div	20		CTAB Extraction + real time PCR	yes	
div	29		CTAB-extraction followed by kit based DNA-pruification	yes	
div	41		in house method		

5.2 Homogeneity

5.2.1 Mixture homogeneity before botteling

Microtracer Homogeneity Test
DLA 04-2019 Sample A

Weight whole sample	3,05	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	14,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,94	36	14,6
2	5,06	42	16,6
3	5,02	36	14,3
4	5,06	43	17,0
5	4,97	42	16,9
6	5,10	45	17,6
7	5,02	33	13,1
8	5,02	41	16,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	39,7	Particles
Standard deviation	3,99	Particles
χ^2 (CHI-Quadrat)	2,80	
Probability	**90**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	15,8	mg/kg
Standard deviation	1,59	mg/kg
rel. Standard deviaton	10,0	%
Horwitz standard deviation	10,6	%
HorRat-value	**0,95**	
Recovery rate	110	%

Microtracer Homogeneity Test
DLA 04-2019 Spiking Level Sample

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	78	30,8
2	4,95	63	25,5
3	5,05	81	32,1
4	5,02	78	31,1
5	4,99	74	29,7
6	5,02	73	29,1
7	5,00	77	30,8
8	5,05	70	27,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	74,2	Particles
Standard deviation	5,39	Particles
χ^2 (CHI-Quadrat)	2,73	
Probability	**91**	%
Recovery rate	131	%

Normal distribution		
Number of samples	8	
Mean	29,6	mg/kg
Standard deviation	2,15	mg/kg
rel. Standard deviaton	7,25	%
Horwitz standard deviation	9,61	%
HorRat-value	**0,75**	
Recovery rate	131	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 04-2019***
PT name	***Allergens IV: Celery, Mustard and Sesame in Spice Salt***
Sample matrix (processing)	***Samples A + B:*** *Matrix (treatment)/ ingredients: Table salt, spices (paprika, pepper, onions), other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each + 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C) Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Celery, Mustard and Sesame (Protein / DNA) Samples A + B: < 500 mg/kg (as food item) Spiking Level Sample: < 500 mg/kg (as food item)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 19th July 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		GREAT BRITAIN
		USA
		CANADA
		CANADA
		ITALY
		Germany
		SPAIN
		ITALY
		ITALY
		Germany
		SWEDEN
		HUNGARY
		GREAT BRITAIN
		CANADA
		Germany
		SERBIA
		SWEDEN
		POLAND
		SWITZERLAND
		FRANCE
		SPAIN
		Germany
		Germany
		SWEDEN
		AUSTRIA
		HUNGARY
		CANADA
		GREAT BRITAIN
		ISRAEL
		ITALY
		FRANCE
		Germany
		GREECE
		GREAT BRITAIN
		GREAT BRITAIN
		PORTUGAL
		SPAIN
		SLOVAKIA
		GREAT BRITAIN
		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

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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

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36. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - 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 (2017) [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]