Evaluation Report
proficiency test

DLA ptAL04 (2020)

Allergens IV:

Celery, Mustard and Sesame

in Tomato Cream Soup (Instant Powder)

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

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EP-Nummer *PT-Number*	DLA ptAL04 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (19 October 2020) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 19 October 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Contents

1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a common in commerce instant soup powder (tomato cream soup) with addition of potato flour. The basic composition of both sample A and sample B was the same (see table 1).
After sieving (mesh 2,0 mm) the basic mixture was homogenized.
Afterwards the **spiked sample B** was produced as follows:
The spiking materials containing the allergenic ingredients celery, mustard and sesame were sieved by means of a centrifugal mill (mesh 250 µm), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 3 additional steps and homogenized in each case until the total quantity had been reached.
The **spiking level sample** was produced with the allergenic compounds above mentioned by multi-stage addition of potato powder (mesh 500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Tomato Cream Soup Powder Ingredients: Tomato powder (37%), starch, sugar, salt, onion powder, rice flour, yeast extract, corn oil, maltodextrin, spices (garlic, pepper), beetroot juice powder / beetroot juice powder, herbs (bay leaves, oregano), flavors Nutrients per 100 g: Fat <5,5 g, Carbohydrates 68 g, Protein 5,5 g, Salt 9,9 g	66,5 g/100 g	65,6 g/100g	-
Potato Flour Nutrients per 100 g: Protein 0g	33,5 g/100 g	33,0 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	1,19 g/100 g	99,8 g/100 g
Celery seed: - as celery* - thereof 20,0% total protein**	-	 36,2 mg/kg 7,24 mg/kg	 31,2 mg/kg 6,24 mg/kg
Mustard, yellow: - as mustard* - thereof 30,6% total protein**	-	 24,5 mg/kg 7,48 mg/kg	 21,1 mg/kg 6,45 mg/kg
Sesame, white: - as Sesame seed* - *thereof 24,5% total protein***		 46,4 mg/kg 11,4 mg/kg	 40,1 mg/kg 9,81 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	<0,2 g/100 g	<0,2 g/100 g

* Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw materials (total nitrogen according to Kjeldahl with F=6,25 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 B and the spiking level sample showed a probability of 89% and 98%. 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 0,9 or 0,7. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

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

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

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

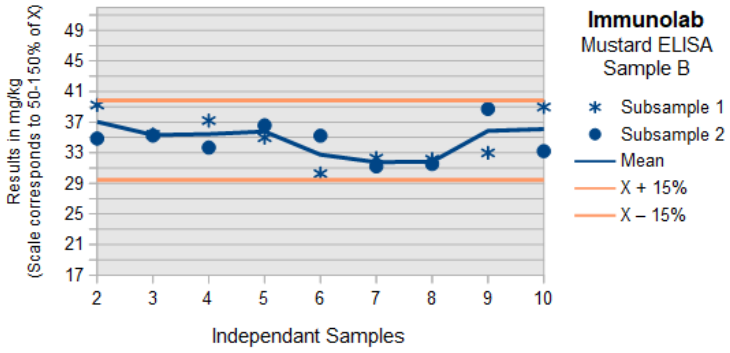
ELISA-Tests: Homogenität Senf / Homogeneity Mustard

Immunolab Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 34,7 ± 1,5 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
2	39,2	34,9	37,0
3	35,4	35,2	35,3
4	37,2	33,7	35,4
5	35,0	36,6	35,8
6	30,3	35,2	32,7
7	32,3	31,2	31,8
8	32,1	31,5	31,8
9	33,0	38,7	35,9
10	39,0	33,2	36,1

General average X	34,7	
SD of sample means Sx	1,98	5,7%
SD within-samples Sw	1,87	5,4%
SD between-samples Ss	1,48	4,3%

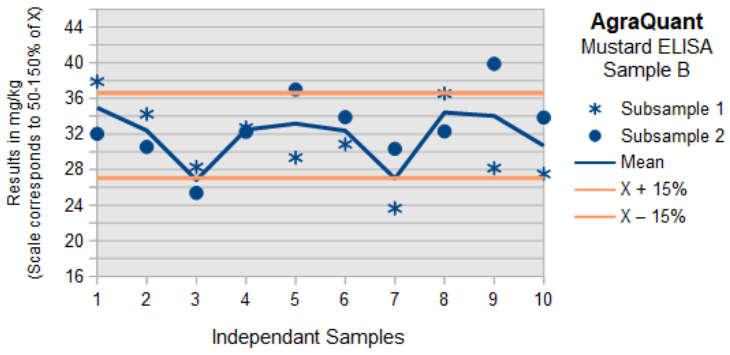


AgraQuant Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 31,8 ± 1,9 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	37,9	32,0	34,9
2	34,2	30,5	32,4
3	28,3	25,4	26,8
4	32,7	32,2	32,5
5	29,3	37,0	33,2
6	30,8	33,9	32,4
7	23,6	30,3	27,0
8	36,5	32,3	34,4
9	28,2	39,9	34,0
10	27,5	33,8	30,7

General average X	31,8	
SD of sample means Sx	2,86	9,0%
SD within-samples Sw	3,02	9,5%
SD between-samples Ss	1,91	6,0%



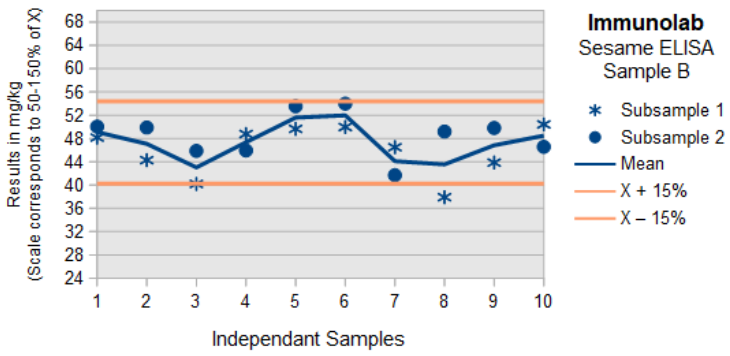
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Immunolab Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 47,3 ± 2,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	48,2	50,0	49,1
2	44,3	49,9	47,1
3	40,2	45,9	43,0
4	48,8	45,9	47,4
5	49,6	53,5	51,6
6	50,0	54,0	52,0
7	46,5	41,7	44,1
8	37,9	49,2	43,5
9	43,9	49,8	46,8
10	50,4	46,6	48,5

General average X	47,3	
SD of sample means Sx	3,12	6,6%
SD w ithin-samples Sw	2,77	5,9%
SD betw een-samples Ss	2,43	5,1%

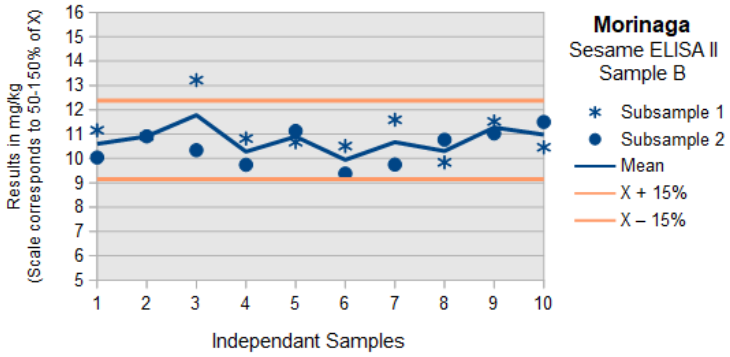


Morinaga Sesame ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame protein 10,8 ± 0,24 mg/kg

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

General average X	10,8	
SD of sample means Sx	0,530	4,9%
SD w ithin-samples Sw	0,667	6,2%
SD betw een-samples Ss	0,243	2,3%

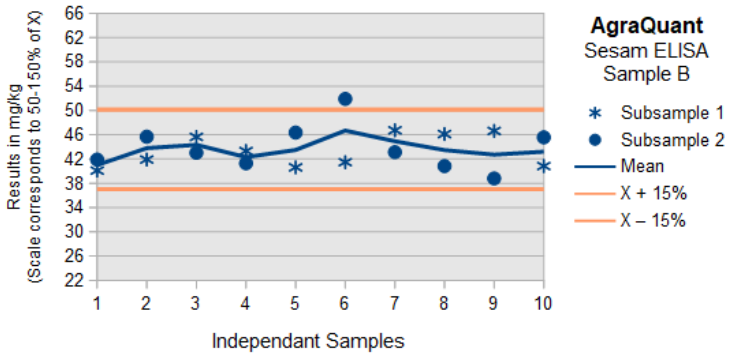


AgraQuant Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 43,6 ± 1,5 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	40,1	41,9	41,0
2	41,9	45,7	43,8
3	45,6	43,0	44,3
4	43,3	41,3	42,3
5	40,6	46,3	43,5
6	41,5	51,9	46,7
7	46,7	43,1	44,9
8	46,1	40,8	43,5
9	46,6	38,8	42,7
10	40,8	45,6	43,2

General average X	43,6	
SD of sample means Sx	1,54	3,5%
SD w ithin-samples Sw	2,71	6,2%
SD betw een-samples Ss	< 1,54	< 3,5%



2.1.2 Stability

The pap samples are preparations preserved with sorbic acid. The stability of the sample material was thus guaranteed during the investigation period under the specified storage conditions.
A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,43 (18,3°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A, B and the spiking level sample were sent to every participating laboratory in the 24th week of 2020. The testing method was optional. The tests should be finished at 21st August 2020 the latest (extended).

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

There are two different samples A and B possibly containing the allergenic parameters Celery, Mustard and Sesame in the range of mg/kg in the matrix of Instant Soup Powder. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing and should be analysed like a normal sample.

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, limit of quantifications, test kit manufacturer and 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 39 participants submitted their results in time.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [25, 26, 27, 28]. It is for this reason that we contrast the results of the present proficiency test with several assigned values.
Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.
For quantitative results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD	RSD_r	RSD_R	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 18 – 37% 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]**	**Recovery**	**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*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

If $U_{(xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.
The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

ELISA results given as **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 (Xpt)	$\boldsymbol{Xpt_{ALL}}$	$\boldsymbol{Xpt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (Xpt)		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σpt *or* $\sigma pt'$		
lower limit of target range ($Xpt - 2\sigma pt$) or ($Xpt - 2\sigma pt'$)°		
upper limit of target range ($Xpt + 2\sigma pt$) or ($Xpt + 2\sigma pt'$)°		
Quotient S*/σpt *or* S*/$\sigma pt'$		
Standard uncertainty $U(Xpt)$		
Number of results in target range		
Percent in target range		

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

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

4.1 Proficiency Test 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		
1	negative		positive		2/2 (100%)	ASU	
11	negative		positive		2/2 (100%)	ASU	
23	positive		negative		0/0 (0%)	ASU	samples interchanged?
28	negative		positive		2/2 (100%)	CEN	
32	negative		positive		2/2 (100%)	CEN	
8	negative		positive		2/2 (100%)	IM	
4	negative		negative		1/2 (50%)	MS	no positive sample detected
3	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA	
15	negative		positive		2/2 (100%)	SFA	
22	negative		positive		2/2 (100%)	SFA	
34	negative		positive		2/2 (100%)	SFA	
21	negative		positive		2/2 (100%)	SFA-4p	
38	negative	<1	positive	41,5	2/2 (100%)	SFA-ID	
10	negative		positive		2/2 (100%)	div	
18	negative		positive		2/2 (100%)	div	
19	negative		positive		2/2 (100%)	div	
24	negative	<1	positive	>1	2/2 (100%)	div	
33a	negative		positive	3,00	2/2 (100%)	div	
33b	negative		positive	7,00	2/2 (100%)	div	
36	negative		positive		2/2 (100%)	div	
37	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	1		20	
Number negative	21		2	
Percent positive	5		91	
Percent negative	95		9	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
CEN = CEN Methoden/ methods
IM = Imegen
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 B.

Quantitative Valuation PCR: Sample B

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

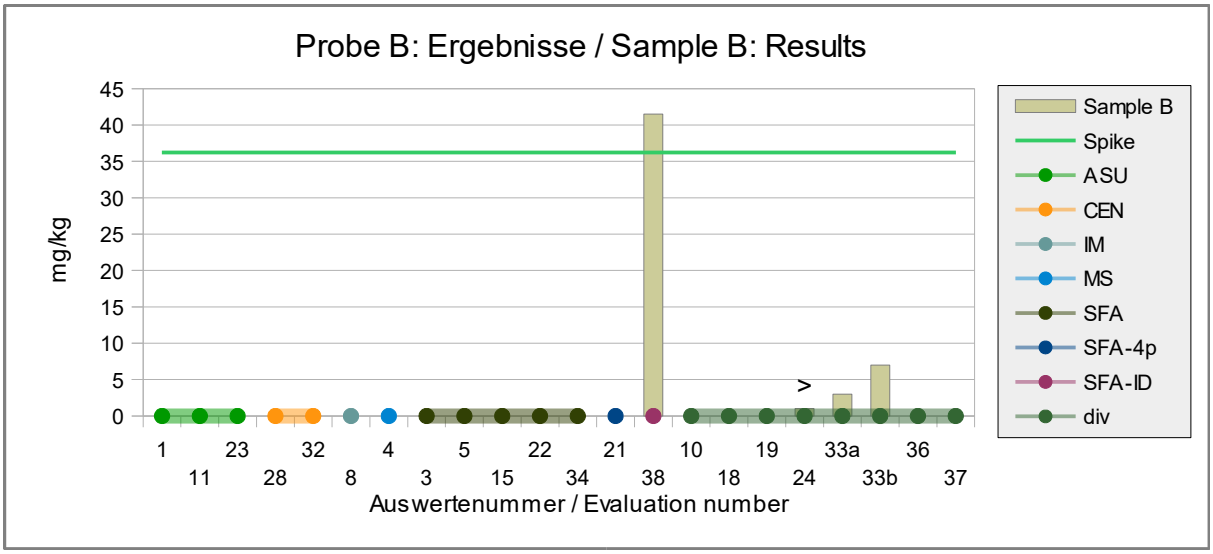


Abb./Fig. 1: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

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	Celery	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positive			ASU	
11	positive			ASU	
23	positive			ASU	
28	positive			CEN	
32	positive			CEN	
8	positive			IM	
4	positive	30		MS	
3	positive			SFA	
5	positive			SFA	
15	positive			SFA	
22	positive			SFA	
34	positive			SFA	
21	positive			SFA-4p	
38	positive	46,8		SFA-ID	
10	positive			div	
18	positive			div	
19	positive			div	
24	positive	>1		div	
33a	positive	2		div	
33b	positive	5		div	
36	positive			div	
37	positive			div	

Number positive	22
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:

ASU = ASU §64 Methode/method
CEN = CEN Methoden/ methods
IM = Imegen
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:
100% positeve results were obtained for the spiking level sample.

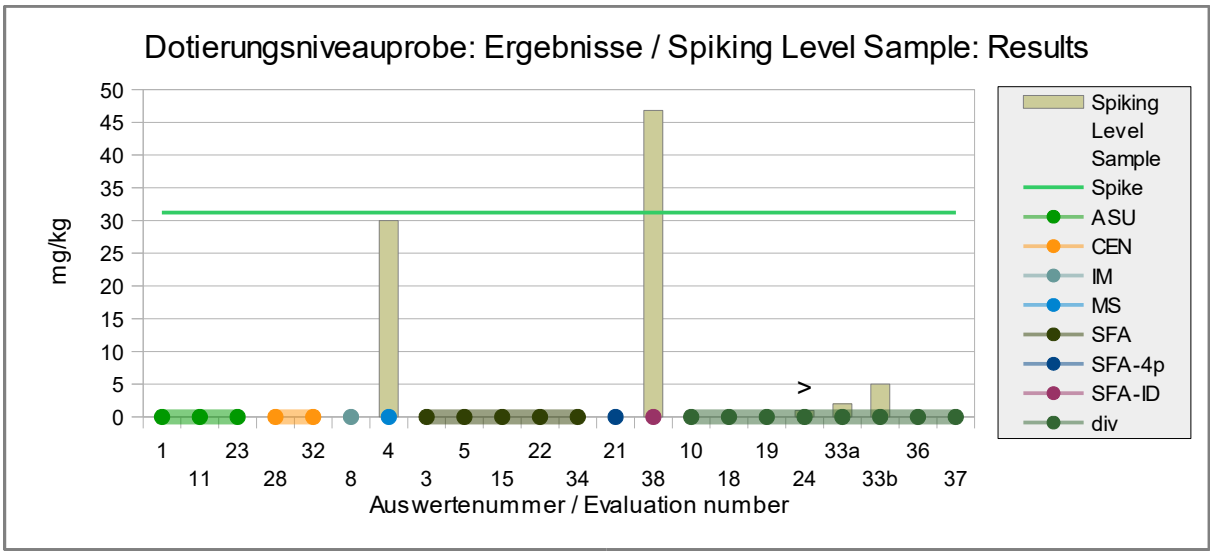


Abb./Fig. 2: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Celery: Spiking Level Sample and Sample B

	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
1							ASU	
11							ASU	
23							ASU	
28							CEN	
32							CEN	
8							IM	
4	30	**96**	-0,15				MS	
3							SFA	
5							SFA	
15							SFA	
22							SFA	
34							SFA	
21							SFA-4p	
38	46,8	**150**	2,0	41,5	**115**	0,58	SFA-ID	
10							div	
18							div	
19							div	
24	>1			>1			div	
33a	2	6	-3,7	3,00	8	-3,7	div	
33b	5	16	-3,4	7,00	19	-3,2	div	
36							div	
37							div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**2**	Number in RA	**1**
Percent in RA	**50**	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
CEN = CEN Methoden/ methods
IM = Imegen
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:

By PCR methods 2 of 4 participants obtained with the spiking level sample a recovery rate within the range of the AOAC-recommendation of 50-150%. For the food matrix sample B one of 3 recovery rates were within this range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

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		
12	negative	<LOD	positive	45,1	2/2 (100%)	AQ	
37	negative	<2	positive	25,8	2/2 (100%)	AQ	
26	negative	<2	positive	28,7	2/2 (100%)	BC	
39	negative	0	positive	22,4	2/2 (100%)	BF	
23	positive	38,9	negative	<2,00	0/0 (0%)	NL-E	samples interchanged?
15	negative	< 2	positive	28,3	2/2 (100%)	OS	
1	negative		positive	105	2/2 (100%)	RS-F	
2	negative	<0,5	positive	>13,5	2/2 (100%)	RS-F	
10	negative	<0,5	positive	36,3	2/2 (100%)	RS-F	
13	negative	<2,5	positive	>13,5	2/2 (100%)	RS-F	
18	negative		positive	37,0	2/2 (100%)	RS-F	
22	negative	<0,5	positive	>13,5	2/2 (100%)	RS-F	
24	negative	<0,5	positive	21,3	2/2 (100%)	RS-F	
25	negative	<0,5	positive	36,7	2/2 (100%)	RS-F	
36	negative	<	positive	8,90	2/2 (100%)	RS-F	
38	negative	<0,5	positive	22,0	2/2 (100%)	RS-F	
11a	negative	<2	positive	15,0	2/2 (100%)	SP	
14	negative	0	positive	33,0	2/2 (100%)	SP	
6	negative	<1	positive	36,0	2/2 (100%)	VT	
7	negative	<2,5	positive	35,0	2/2 (100%)	VT	
11b	negative	<2,5	positive	47,0	2/2 (100%)	VT	
16	negative		positive	36,9	2/2 (100%)	VT	
17	negative	<1,0	positive	35,7	2/2 (100%)	VT	
27	negative	ND	positive	27,3	2/2 (100%)	VT	
30	negative	<2,5	positive	36,0	2/2 (100%)	VT	
32	negative		positive	41,0	2/2 (100%)	VT	
34	negative	<8,2	positive	215	2/2 (100%)	VT	result converted °

° calculation p. 19

	Sample A		Sample B	
Number positive	1		26	
Number negative	26		1	
Percent positive	4		96	
Percent negative	96		4	
Consensus value	negative		positive	

Methods:

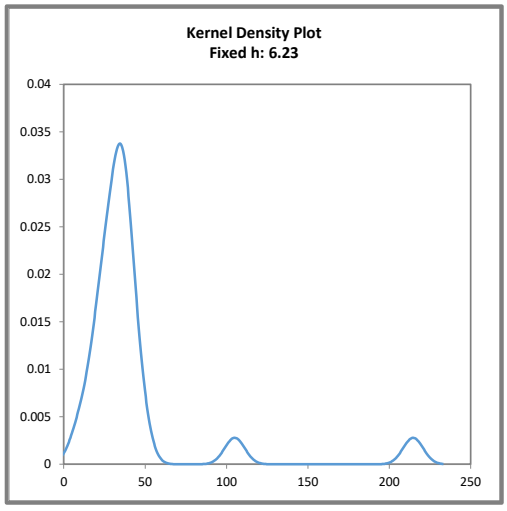
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
NL-E = nutriLinia®E Allergen-ELISA
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
12	45,1	1,4			AQ	
37	25,8	-0,89			AQ	
26	28,7	-0,55			BC	
39	22,4	-1,3			BF	
23	<2,00				NL-E	
15	28,3	-0,60			OS	
1	105	8,6			RS-F	outlier $Xpt_{RS\text{-}F}$ excluded
2	>13,5				RS-F	
10	36,3	0,37	1,4		RS-F	
13	>13,5				RS-F	
18	37,0	0,45	1,5		RS-F	
22	>13,5				RS-F	
24	21,3	-1,4	-0,85		RS-F	
25	36,7	0,42	1,4		RS-F	
36	8,90	-2,9	-2,7		RS-F	
38	22,0	-1,4	-0,74		RS-F	
11a	15,0	-2,2			SP	
14	33,0	-0,03			SP	
6	36,0	0,33		-0,09	VT	
7	35,0	0,21		-0,19	VT	
11b	47,0	1,7		1,1	VT	
16	36,9	0,44		0,01	VT	
17	35,7	0,30		-0,12	VT	
27	27,3	-0,71		-1,0	VT	
30	36,0	0,33		-0,08	VT	
32	41,0	0,93		0,46	VT	
34	215	22			VT	result converted ° outlier Xpt_{VT} excluded

° calculation p. 19



Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
NL-E = nutriLinia®E Allergen-ELISA
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
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 two secondary peaks at 105 mg/kg and 215 mg/kg, due to two outliers (methods RS-F and VT).

Characteristics: Quantitative evaluation ELISA Mustard

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*	**Method VT** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$	$X_{pt_{METHOD\ VT}}$
Number of results	23	6°	8°
Number of outliers	-	1	1
Mean	42,4	27,0	36,9
Median	35,7	29,2	36,0
Robust Mean (X_{pt})	**33,2**	**27,0**	**36,8**
Robust standard deviation (S*)	**11,0**	**13,1**	**4,89**
Target range:			
Target standard deviation σ_{pt}	**8,31**	**6,76**	**9,19**
lower limit of target range	**16,6**	**13,5**	**18,4**
upper limit of target range	**49,9**	**40,6**	**55,1**
Quotient S/σ_{pt}*	*1,3*	*1,9*	*0,53*
Standard uncertainty $U_{(Xpt)}$	*2,87*	*6,68*	*2,16*
Results in the target range	*19*	*5*	*8*
Percent in the target range	*83*	*83*	*100*

° *without results no. 1 and no. 34, respectively (excluded)*

Methods:
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 evaluations of all methods, methods RS-F and VT showed a normal to low variability of results, with quotients 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 136%, 110% and 150% of the spiking level of Mustard to sample B within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA for Mustard" p.35).

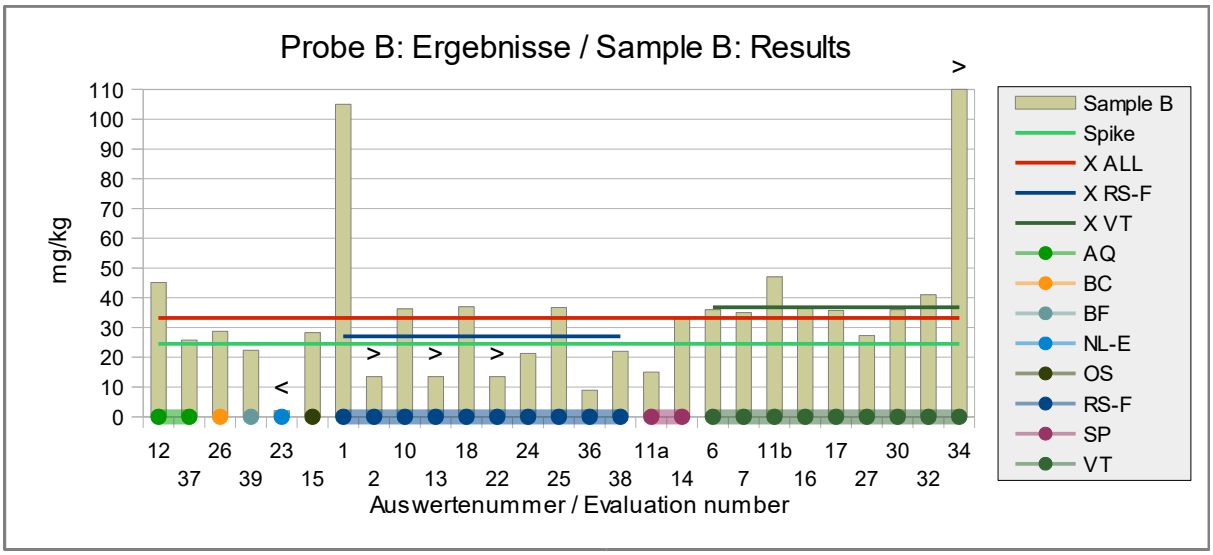


Abb./Fig. 4: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

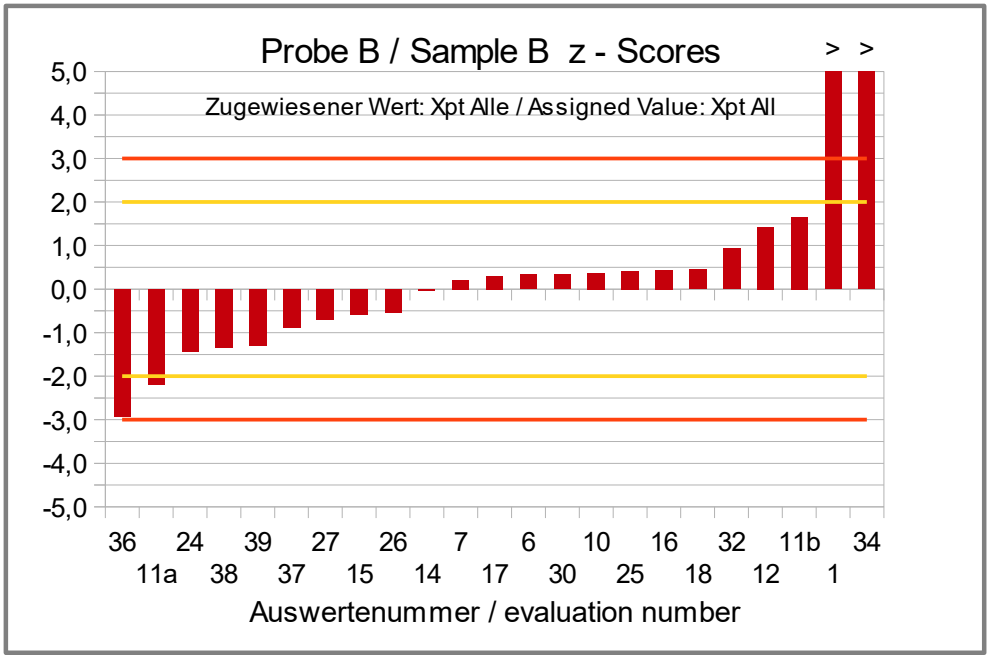


Abb./Fig. 5:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

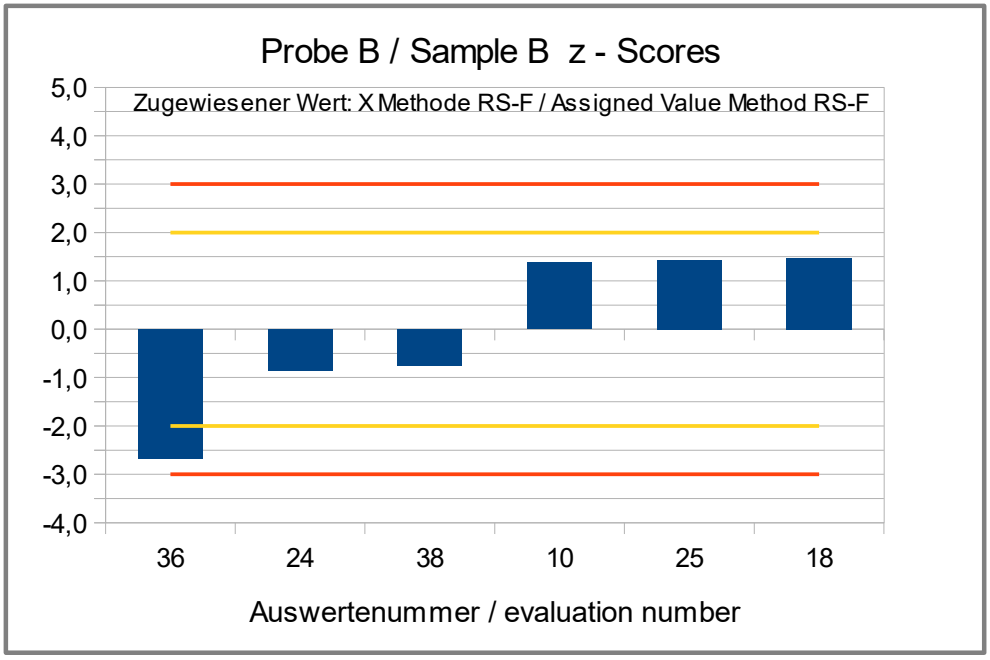


Abb./Fig. 6:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen)

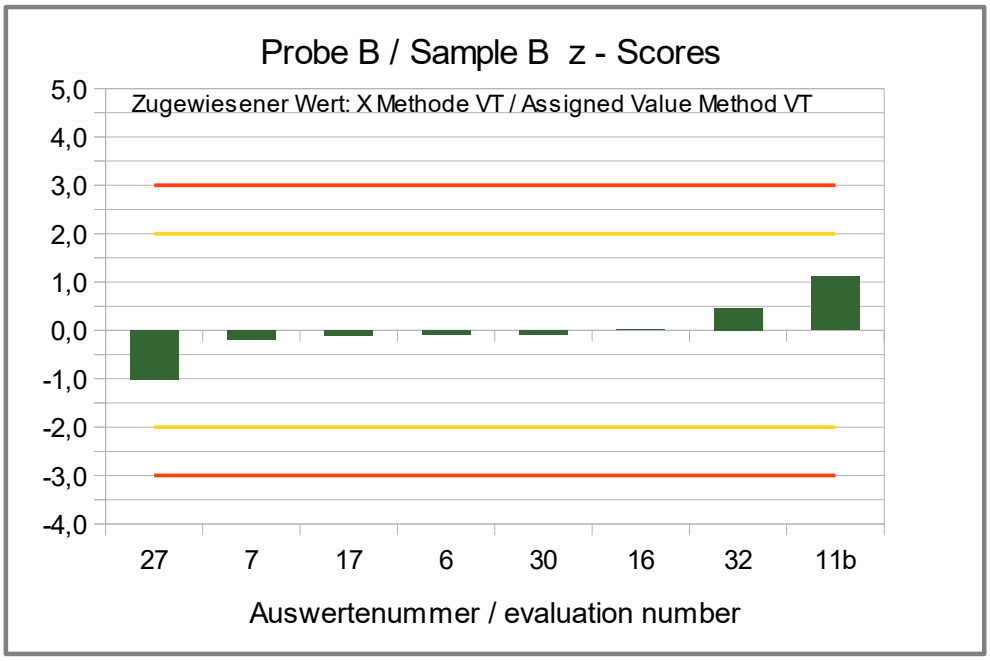
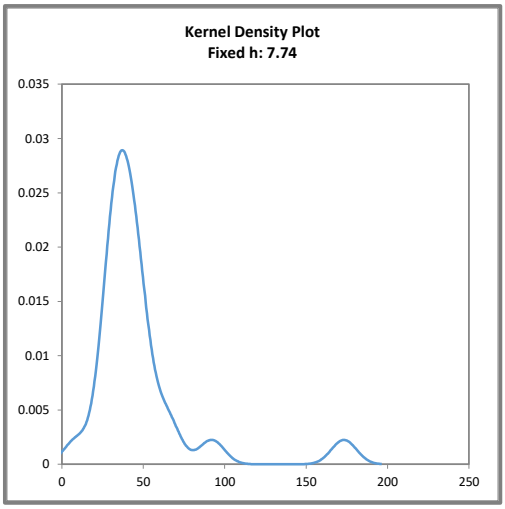


Abb./Fig. 7:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Spiking Level Sample	z-Score Xpt_{ALL}	z'-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
12	44,5	0,31			AQ	
37	62,0	2,0			AQ	
26	31,6	-0,94			BC	
39	47,8	0,63			BF	
23	38,7	-0,25			NL-E	
15	30,6	-1,0			OS	
1	92,0	4,9			RS-F	outlier $Xpt_{RS\text{-}F}$ excluded
2	>13,5				RS-F	
10	66,4	2,4	2,1		RS-F	
13	>13,5				RS-F	
18	29,0	-1,2	-0,51		RS-F	
22					RS-F	
24	36,7	-0,44	0,04		RS-F	
25	44,2	0,28	0,56		RS-F	
36	8,90	-3,1	-1,9		RS-F	
38	32,2	-0,88	-0,29		RS-F	
11a	48,0	0,65			SP	
14	44,0	0,27			SP	
6	48,7	0,72		1,4	VT	
7	32,0	-0,90		-0,48	VT	
11b	42,0	0,07		0,62	VT	
16	35,7	-0,54		-0,08	VT	
17					VT	
27	27,6	-1,3		-0,97	VT	
30	35,0	-0,61		-0,15	VT	
32	35,0	-0,61		-0,15	VT	
34	173	13			VT	result converted ° outlier Xpt_{VT} excluded

° calculation p. 19



Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
NL-E = nutriLinia®E Allergen-ELISA
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with two secondary peaks at approx. 92 mg/kg and 173 mg/kg, due to two outliers (methods RS-F and VT).

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	23	6°	7°
Number of outliers	-	1	1
Mean	47,2	36,2	36,6
Median	38,7	34,5	35,0
Robust Mean (X_{pt})	**41,3**	**36,2**	**36,4**
Robust standard deviation (S*)	**13,2**	**21,5**	**7,41**
Target range:			
Target standard deviation σ_{pt} or $\sigma_{pt'}$	**10,3**	**14,2**	**9,10**
lower limit of target range	**20,6**	**7,82**	**18,2**
upper limit of target range	**61,9**	**64,7**	**54,6**
Quotient S/σ_{pt} or S*/$\sigma_{pt'}$*	*1,3*	*1,5*	*0,81*
Standard uncertainty $U_{(Xpt)}$	*3,44*	*10,9*	*3,50*
Results in the target range	*19*	*5*	*7*
Percent in the target range	*83*	*83*	*100*

° *without results no. 1and no. 34 (excluded)*

Methods:
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 VT showed a normal to low variability of results, with quotients S/σ_{pt} below 2,0. The distribution of results from method RS-F showed a slightly increased variability with a S*/σ_{pt} >2,0. Therefore the evaluation was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ wase then below 2,0.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods or for method RS-F slightly higher (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 196%, 172% and 173% of the spiking level of mustard to the spiking level sample and were thus above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA for Mustard" p.35).

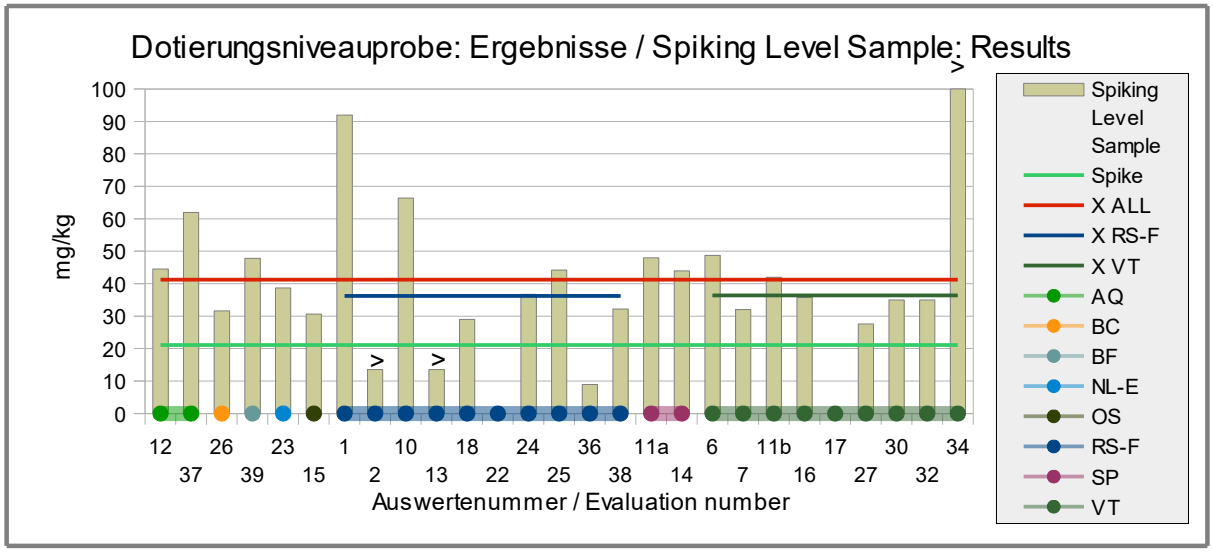


Abb./Fig. 9: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

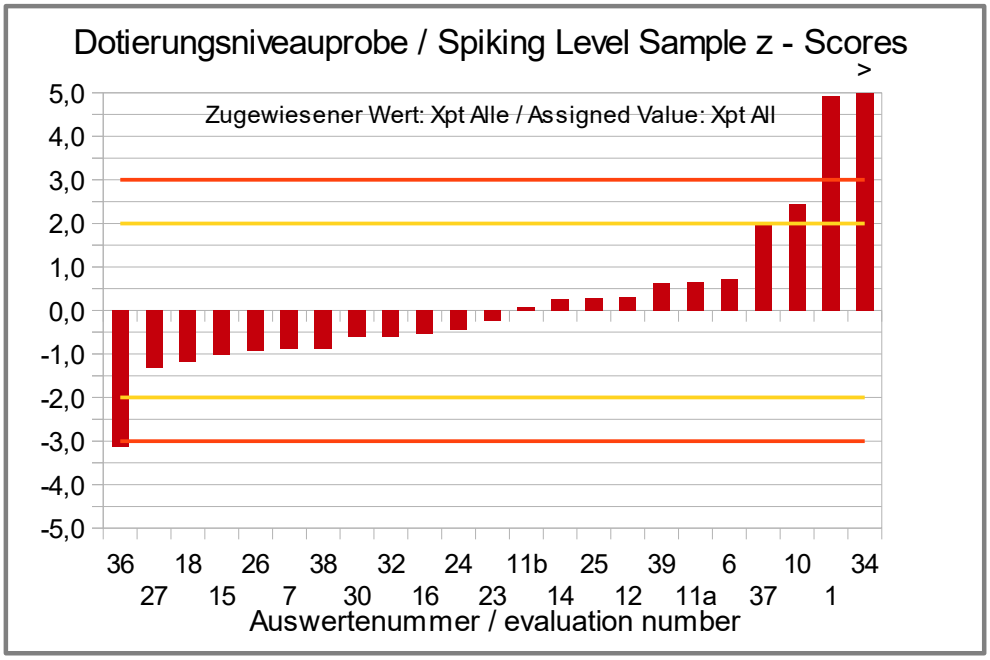


Abb./Fig. 10:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

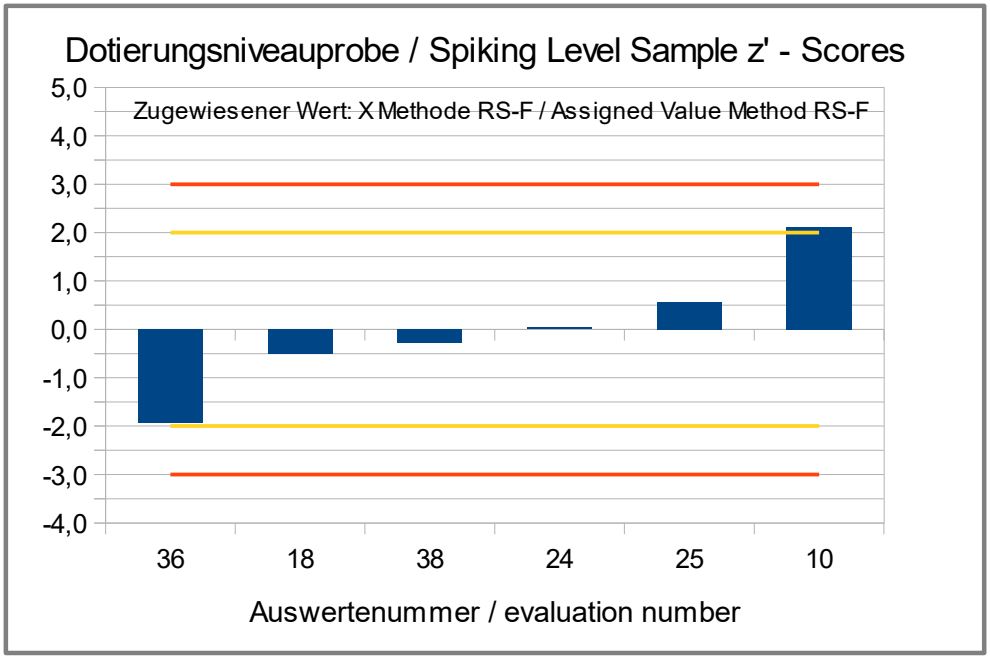


Abb./Fig. 11:
z'-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

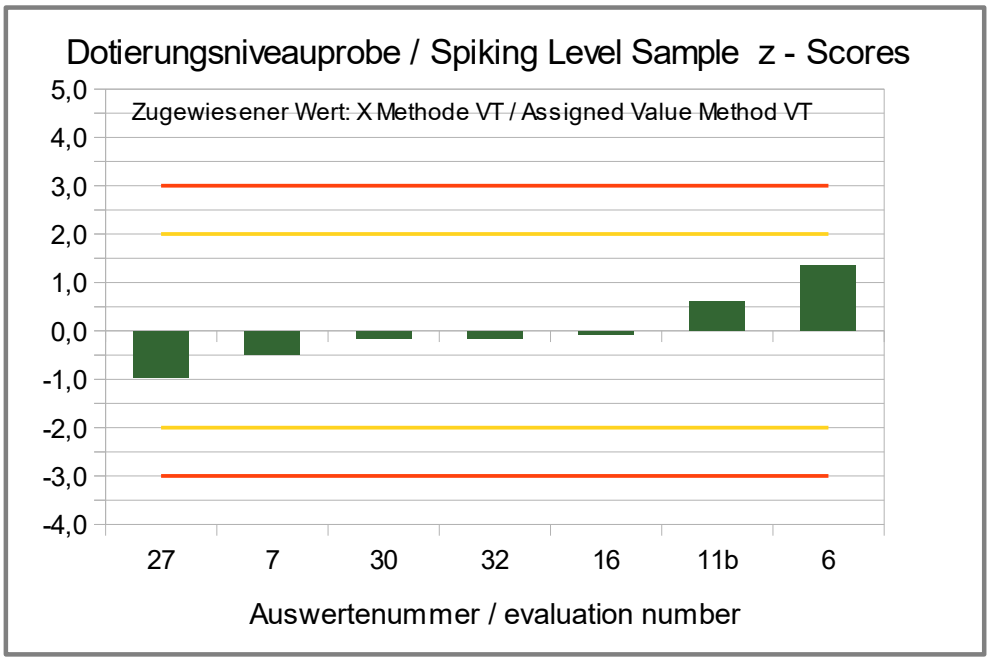


Abb./Fig. 12:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
12	44,5	211	4,4	45,1	184	3,4	AQ	
37	62,0	294	7,8	25,8	**105**	0,21	AQ	
26	31,6	**150**	2,0	28,7	**117**	0,69	BC	
39	47,8	227	5,1	22,4	**91**	-0,34	BF	
23	38,7	183	3,3	<2,00			NL-E	
15	30,6	**145**	1,8	28,3	**115**	0,62	OS	
1	92,0	436	13	105	429	13,1	RS-F	
2	>13,5			>13,5			RS-F	
10	66,4	315	8,6	36,3	**148**	1,9	RS-F	
13	>13,5			>13,5			RS-F	
18	29,0	**137**	1,5	37,0	151	2,0	RS-F	
22				>13,5			RS-F	
24	36,7	174	3,0	21,3	**87**	-0,52	RS-F	
25	44,2	209	4,4	36,7	**150**	2,0	RS-F	
36	8,90	42	-2,3	8,90	36	-2,5	RS-F	
38	32,2	153	2,1	22,0	**90**	-0,41	RS-F	
11a	48,0	227	5,1	15,0	**61**	-1,6	SP	
14	44,0	209	4,3	33,0	**135**	1,4	SP	
6	48,7	231	5,2	36,0	**147**	1,9	VT	
7	32,0	152	2,1	35,0	**143**	1,7	VT	
11b	42,0	199	4,0	47,0	192	3,7	VT	
16	35,7	169	2,8	36,9	151	2,0	VT	
17				35,7	**146**	1,8	VT	
27	27,6	**131**	1,2	27,3	**111**	0,46	VT	
30	35,0	166	2,6	36,0	**147**	1,9	VT	
32	35,0	166	2,6	41,0	167	2,7	VT	
34	173	821	29	215	876	31	VT	result converted °

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	4	Number in RA	15
Percent in RA	17	Percent in RA	65

* 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
NL-E = nutriLinia®E Allergen-ELISA
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

For the spiking level sample 17% (4) 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 B 65% (15) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

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		
11	negative		positive		2/2 (100%)	ASU	
23	positive		negative		0/0 (0%)	ASU	samples interchanged?
35	negative		positive		2/2 (100%)	ASU	
32	negative		positive		2/2 (100%)	CEN	
36	negative		positive		2/2 (100%)	GR	
4	negative		positive	10,0	2/2 (100%)	MS	
3	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA	
8	negative		positive		2/2 (100%)	SFA	
15	negative		positive		2/2 (100%)	SFA	
38	negative	<1	positive	58,0	2/2 (100%)	SFA	
21	negative		positive		2/2 (100%)	SFA-4p	
10	negative		positive		2/2 (100%)	div	
19	positive		positive		1/2 (50%)	div	
28a	negative		positive		2/2 (100%)	div	
28b	negative		negative		1/2 (50%)	div	detection of brown and black mustard only
33a	negative		positive	12,0	2/2 (100%)	div	
33b	negative		positive	12,0	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	2		16	
Number negative	16		2	
Percent positive	11		89	
Percent negative	89		11	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
CEN = CEN Methode/method
GR = SPECIALfinder Assay, real time PCR, Generon
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 B.

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 B

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

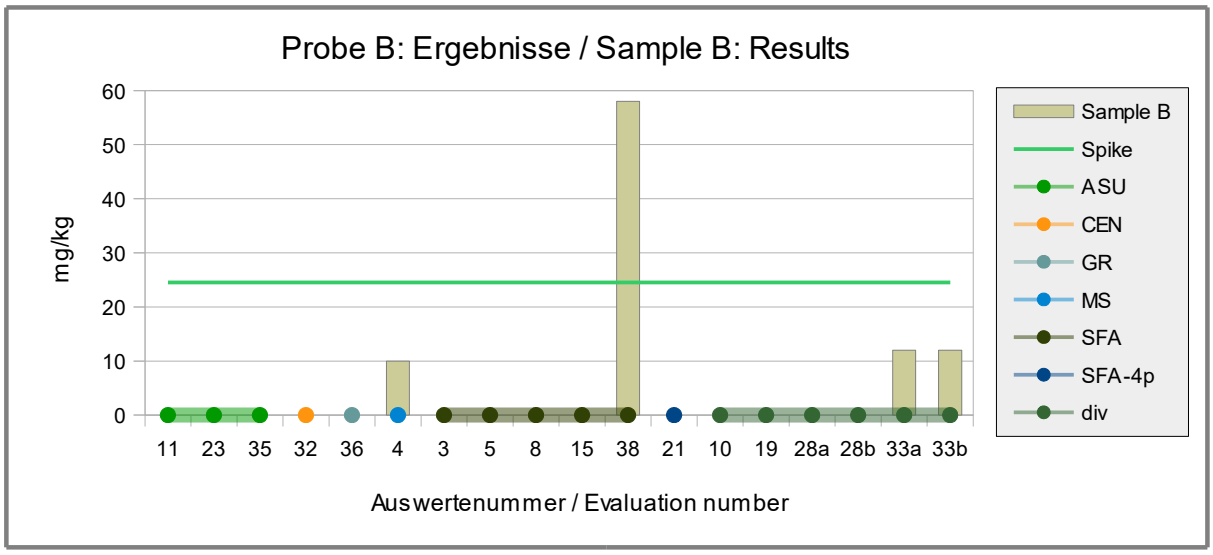


Abb./Fig. 13: PCR Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

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]			
11	positive			ASU	
23	positive			ASU	
35	positive			ASU	
32	positive			CEN	
36	positive			GR	
4	positive	10,0		MS	
3	positive			SFA	
5	positive			SFA	
8	positive			SFA	
15	positive			SFA	
38	positive	8,59		SFA	
21	positive			SFA-4p	
10	positive			div	
19	negative			div	
28a	positive			div	
28b	negative			div	detection of brown and black mustard only
33a	positive	7,00		div	
33b	positive	8,00		div	

Number positive	16
Number negative	2
Percent positive	89
Percent negative	11
Consensus value	positive

Methods:

ASU = ASU §64 Methode/method
CEN = CEN Methode/method
GR = SPECIALfinder Assay, real time PCR, Generon
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:
For the spiking level sample 100% positive results were obtained.

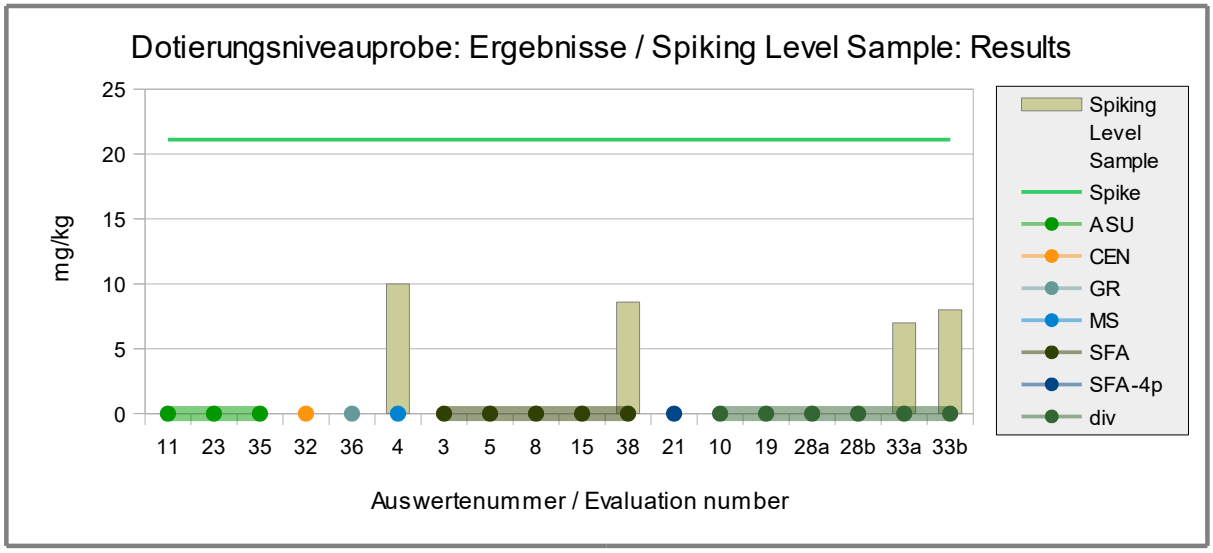


Abb./Fig. 14: PCR-Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Mustard: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
11							ASU	
23							ASU	
35							ASU	
32							CEN	
36							GR	
4	10,0	47	-2,1	10,0	41	-2,4	MS	
3							SFA	
5							SFA	
8							SFA	
15							SFA	
38	8,59	41	-2,4	58,0	237	5,5	SFA	
21							SFA-4p	
10							div	
19							div	
28a							div	
28b							div	
33a	7,00	33	-2,7	12,0	49	-2,0	div	
33b	8,00	38	-2,5	12,0	49	-2,0	div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

CEN = CEN Methode/method

GR = SPECIALfinder Assay, real time PCR, Generon

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:

None of the participants optained for the spiking level sample or for the spiked food matrix sample B a recovery rate by PCR methods within the range of the AOAC-recommendation of 50-150%.
With one exception the results are slightly below the target range.
The related z-scores are based on the target standard deviation of 25%.

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		
12	negative	<LOD	positive	25,4	2/2 (100%)	AQ	
37	negative	<2	positive	43,0	2/2 (100%)	AQ	
20	negative	<2	positive	36,9	2/2 (100%)	BC	
26	negative	<2	positive	42,7	2/2 (100%)	BC	
39	negative	0	positive	13,9	2/2 (100%)	BF	
7a	negative	<1,0	positive	73,5	2/2 (100%)	ES-I	result converted °
27	negative	ND	positive	60,4	2/2 (100%)	ES-I	result converted °
17	negative	<0,510	positive	49,4	2/2 (100%)	ES-II	result converted °
33	negative		positive	41,0	2/2 (100%)	NL	
1	negative		positive	21,0	2/2 (100%)	RS-F	
2	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
7b	negative	<2,5	positive	110	2/2 (100%)	RS-F	
8	negative	<2,5	positive	93,6	2/2 (100%)	RS-F	
9	negative		positive	138	2/2 (100%)	RS-F	
10	negative	<2,4	positive	135	2/2 (100%)	RS-F	
13	negative	<2,5	positive	190	2/2 (100%)	RS-F	
15	negative	< 2,5	positive	>20	2/2 (100%)	RS-F	
16	negative		positive	121	2/2 (100%)	RS-F	
18	negative		positive	170	2/2 (100%)	RS-F	
22	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
23	positive	100,6	negative	<2,5	0/0 (0%)	RS-F	samples interchanged?
24	negative	<2,5	positive	151	2/2 (100%)	RS-F	
29	negative	<2,5	positive	125	2/2 (100%)	RS-F	
31	negative	<2,5	positive	130	2/2 (100%)	RS-F	
32	negative		positive	179	2/2 (100%)	RS-F	
34	negative	<2,5	positive	144	2/2 (100%)	RS-F	
35	negative	< 2,5	positive	157	2/2 (100%)	RS-F	
36	negative	<	positive	135	2/2 (100%)	RS-F	
38	negative	<2,5	positive	127	2/2 (100%)	RS-F	
11	negative	<2	positive	26,0	2/2 (100%)	SP	
14	negative	0	positive	47,0	2/2 (100%)	SP	
30	negative	<2,0	positive	44,0	2/2 (100%)	SP	
6	negative	<1	positive	201	2/2 (100%)	VT	

° calculation p. 19

	Sample A		Sample B	
Number positive	1		32	
Number negative	32		1	
Percent positive	3		97	
Percent negative	97		3	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ES-I = ELISA-Systems, new
ES-II = ELISA-Systems
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. For sample B a positive result was obtained with the method VT (Veratox).

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Sesame	z-Score Xpt_{43}	z-Score Xpt_{137}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]					
12	25,4	-1,6			AQ	
37	43,0	0,14			AQ	
20	36,9	-0,45			BC	
26	42,7	0,11			BC	
39	13,9	-2,7			BF	
7a	73,5	3,1			ES-I	result converted °
27	60,4	1,8			ES-I	result converted °
17	49,4	0,75			ES-II	result converted °
33	41,0	-0,06			NL	
1	21,0		-3,4	-3,4	RS-F	
2	>20				RS-F	
7b	110		-0,86	-0,78	RS-F	
8	93,6		-1,3	-1,3	RS-F	
9	138		-0,06	0,04	RS-F	
10	135		-0,16	-0,06	RS-F	
13	190		1,4	1,6	RS-F	
15	20				RS-F	
16	121		-0,56	-0,47	RS-F	
18	170		0,85	0,97	RS-F	
22	>20				RS-F	
23	<2,5				RS-F	
24	151		0,29	0,40	RS-F	
29	125		-0,43	-0,34	RS-F	
31	130		-0,29	-0,20	RS-F	
32	179		1,1	1,2	RS-F	
34	144		0,10	0,20	RS-F	
35	157		0,48	0,59	RS-F	
36	135		-0,16	-0,06	RS-F	
38	127		-0,37	-0,27	RS-F	
11	26,0	-1,5			SP	
14	47,0	0,52			SP	
30	44,0	0,23			SP	
6	201		1,7		VT	

° calculation p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ES-I = ELISA-Systems, new
ES-II = ELISA-Systems
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

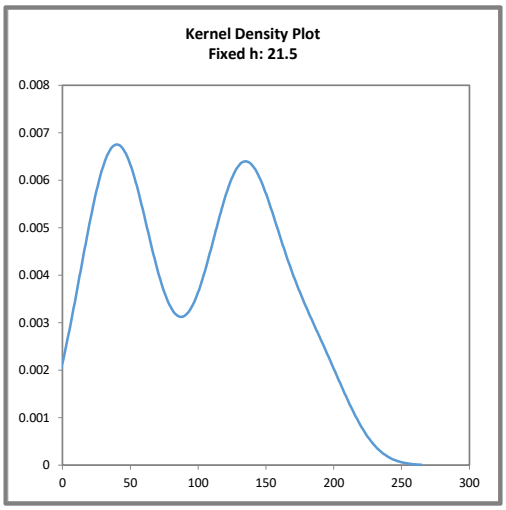


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows two peaks at approx. 42 mg/kg and 140 mg/kg, both with a nearly symmetrical distribution of results.
The higher values ("peak 140") are due to results of the methods RS-F and VT and were therefore evaluated separately.

Characteristics: Quantitative evaluation ELISA Sesame

Sample B

Statistic Data	**Methods Peak 42** *[mg/kg]*	**Methods Peak 140** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{42}}$	$X_{pt_{140}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	12	17	16
Number of outliers	0	-	-
Mean	41,9	137	133
Median	42,9	135	135
Robust Mean (X_{pt})	**41,6**	**140**	**137**
Robust standard deviation (S*)	**15,5**	**34,2**	**30,5**
Target range:			
Target standard deviation σ_{pt}	**10,4**	**35,1**	**34,2**
lower limit of target range	**20,8**	**70,1**	**68,4**
upper limit of target range	**62,4**	**210**	**205**
Quotient S/σ_{pt}*	*1,5*	*0,98*	*0,89*
Standard uncertainty $U_{(Xpt)}$	*5,60*	*10,4*	*9,53*
Results in the target range	*10*	*16*	*15*
Percent in the target range	*83*	*94*	*94*

Methods:
Peak 42 = AgraQuant, BioCheck, BioFront Technologies, ELISA Systems (new), ELISA Systems, nutriLinia®, SensiSpec
Peak 140 = Ridascreen® Fast, Veratox
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a bimodal distribution of results. Therefore no joint evaluation of all methods was carried out, but an evaluation of the methods that are assigned to "peak 42" and another assigned to "peak 140" (Assignment see above below the table).
The distributions of the results of peak 42 and peak 140 as well as of method RS-F showed a normal to low variability of results, with a quotient S/σpt below 2,0 each.*
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 89%, 302% and 294% of the spiking level of sesame to sample B and thus out of the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates ELISA of Sesame" p.52).

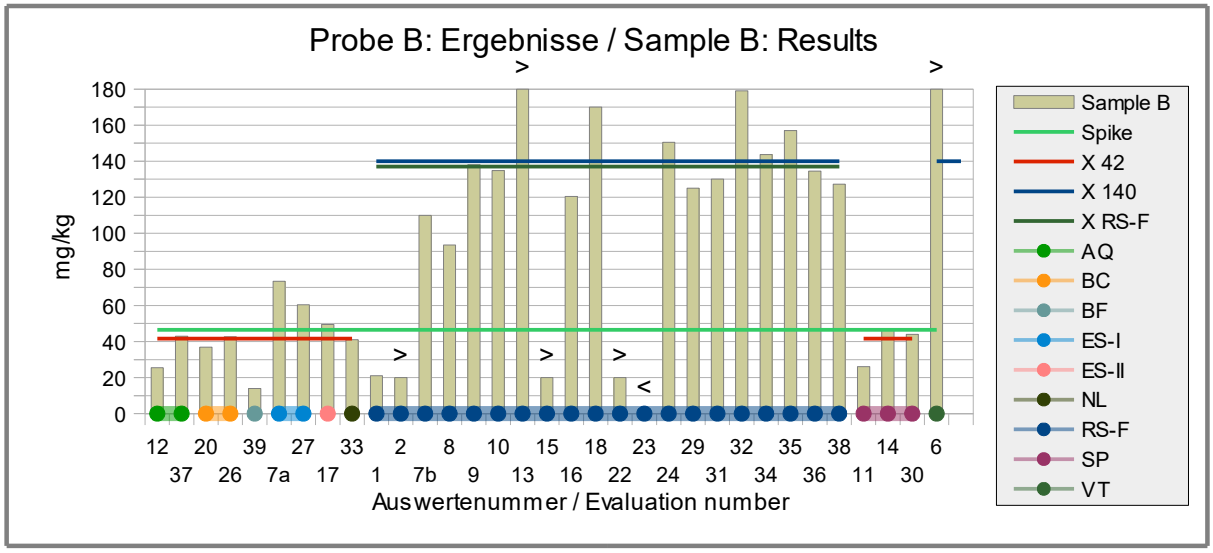


Abb./Fig. 16: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 42"
blue line = Assigned value robust mean all results of "peak 140"
darkgreen line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

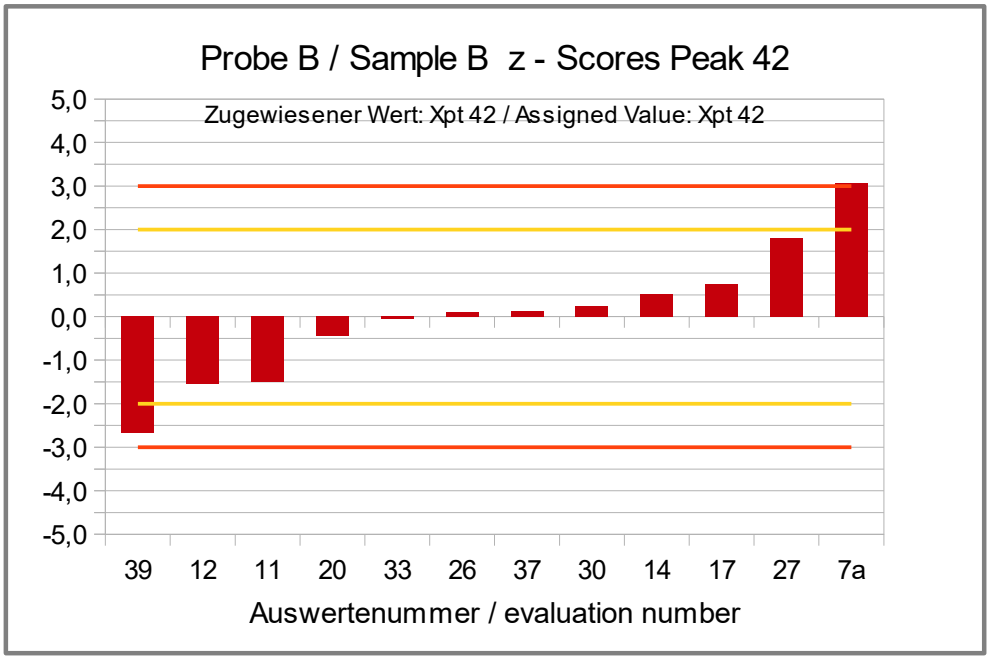


Abb./Fig. 17:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 42

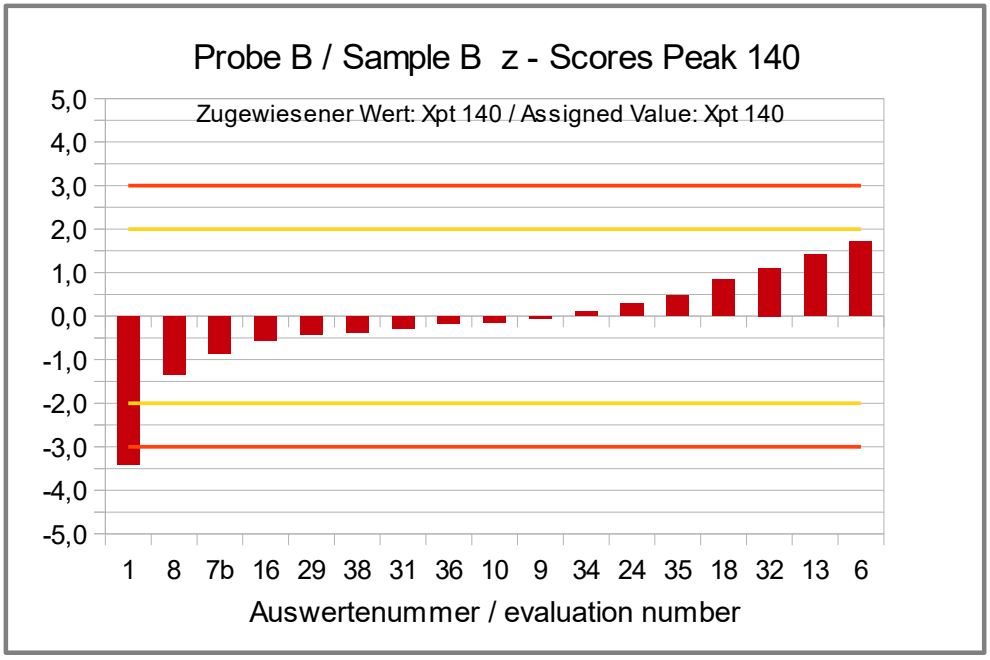


Abb./Fig. 18:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 140

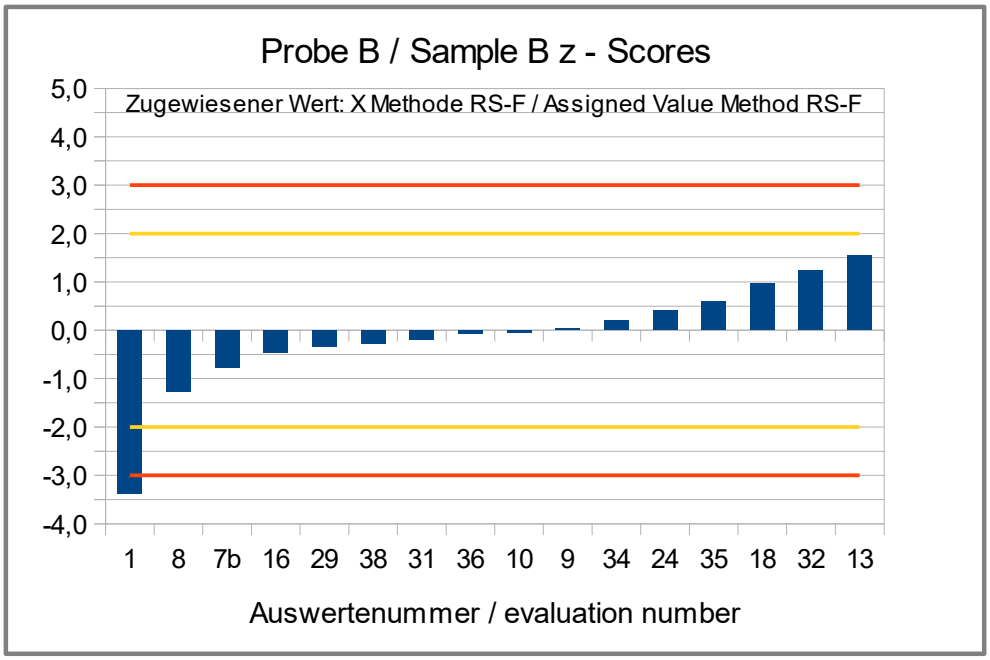


Abb./Fig. 19:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative Valuation of results: Spiking level sample

Evaluation number	Sesame	z-Score Xpt_{37}	z-Score Xpt_{104}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]					
12	18,9	-1,8			AQ	
37	33,5	-0,16			AQ	
20	32,3	-0,30			BC	
26	34,9	0,00			BC	
39	47,9	1,5			BF	
7a	73,5	4,4			ES-I	result converted °
27	61,2	3,0			ES-I	result converted °
17					ES-II	
33	38,0	0,36			NL	
1	25,0		-3,2	-3,2	RS-F	
2	>20				RS-F	
7b	120		-0,11	0,00	RS-F	
8	86,7		-1,2	-1,1	RS-F	
9	100		-0,76	-0,67	RS-F	
10	96,0		-0,89	-0,80	RS-F	
13	147		0,76	0,90	RS-F	
15	>20				RS-F	
16	120		-0,12	-0,02	RS-F	
18	174		1,6	1,8	RS-F	
22					RS-F	
23	103		-0,66	-0,56	RS-F	
24	131		0,26	0,38	RS-F	
29	107		-0,54	-0,44	RS-F	
31	130		0,22	0,34	RS-F	
32	151		0,90	1,0	RS-F	
34	91,7		-1,0	-0,94	RS-F	
35	160		1,2	1,3	RS-F	
36	96,5		-0,87	-0,78	RS-F	
38	160		1,2	1,3	RS-F	
11	22,0	-1,5			SP	
14	42,0	0,81			SP	
30	34,0	-0,10			SP	
6	204		2,6		VT	

° calculation p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
ES-I = ELISA-Systems, new
ES-II = ELISA-Systems
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

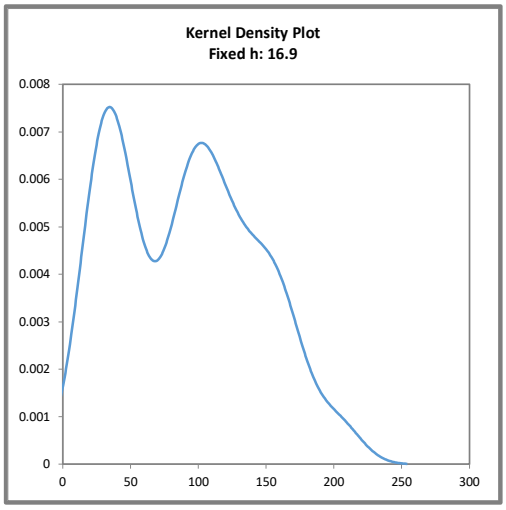


Abb. / Fig. 20:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows two peaks at approx. 35 mg/kg and 123 mg/kg, both with a nearly symmetrical distribution of results. The second peak has a slight shoulder at approx. 155 mg/kg.
The higher values ("peak 123") are due to results of the methods RS-F and VT and were therefore evaluated separately.

Characteristics: Quantitative evaluation ELISA Sesame

Spiking Level Sample

Statistic Data	**Methods Peak 35** *[mg/kg]*	**Methods Peak 123** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{35}}$	$X_{pt_{123}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	11	18	17
Number of outliers	0	0	0
Mean	39,8	122	118
Median (X_{pt})	**34,9** [+]	120	120
Robust Mean (X_{pt})	38,8	**123** [+]	**120** [+]
Robust standard deviation (S*)	**15,8**	**36,7**	**33,7**
Target range:			
Target standard deviation σ_{pt}	**8,73**	**30,8**	**30,0**
lower limit of target range	**17,5**	**61,7**	**60,0**
upper limit of target range	**52,4**	**185**	**180**
Quotient S/σ_{pt}*	*1,8*	*1,2*	*1,1*
Standard uncertainty $U_{(Xpt)}$	*5,97*	*10,8*	*10,2*
Results in the target range	*9*	*16*	*16*
Percent in the target range	*82*	*89*	*94*

[+] *Assigned value (Xpt)*

Method:
Peak 35 = AgraQuant, BioCheck, BioFront Technologies, ELISA Systems (new), ELISA Systems, nutriLinia®, SensiSpec
Peak 123 = Ridascreen® Fast, Veratox
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a bimodal distribution of results. Therefore no joint evaluation of all methods was carried out, but an evaluation of the methods that are assigned to "peak 35" and another assigned to "peak 123" (Assignment see above below the table).
The distributions of the results of peak 35 and peak 123 as well as of method RS-F showed a normal to low variability of results, with a quotient S/σpt below 2,0 each.*
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 median and robust means of the evaluations were 87%, 308% and 299% 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.52).

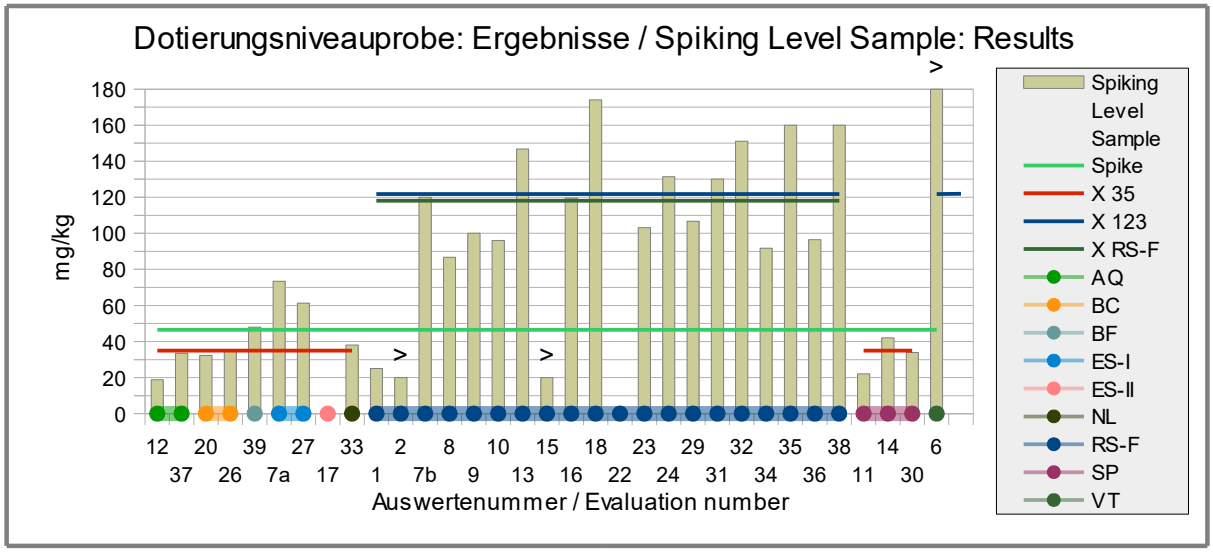


Abb./Fig. 21: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 35"
blue line = Assigned value robust mean all results of "peak 123"
darkgreen line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

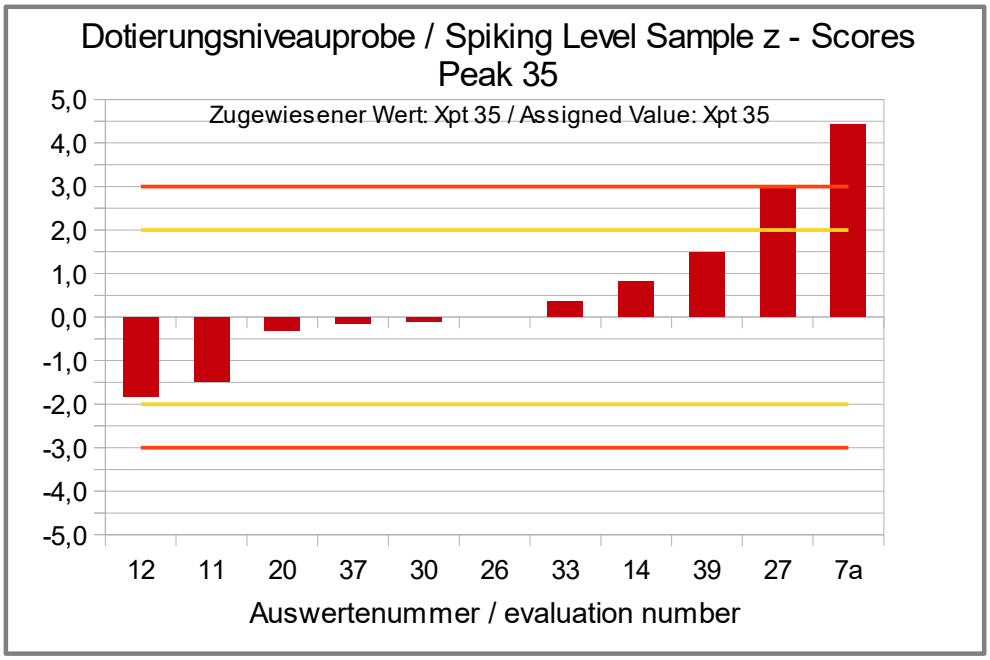


Abb./Fig. 22:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 35

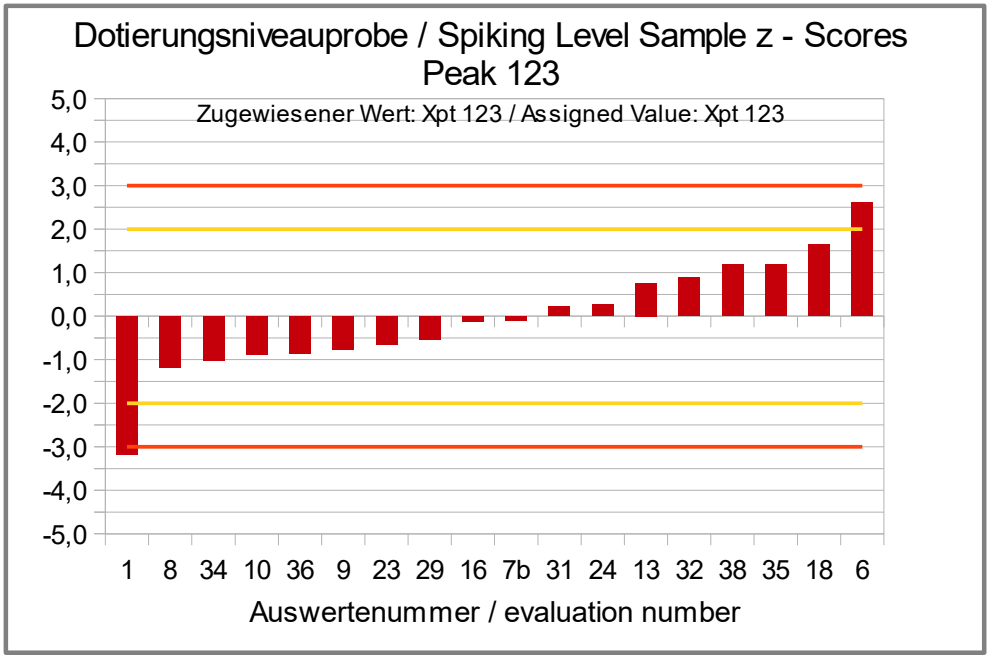


Abb./Fig. 23:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 123

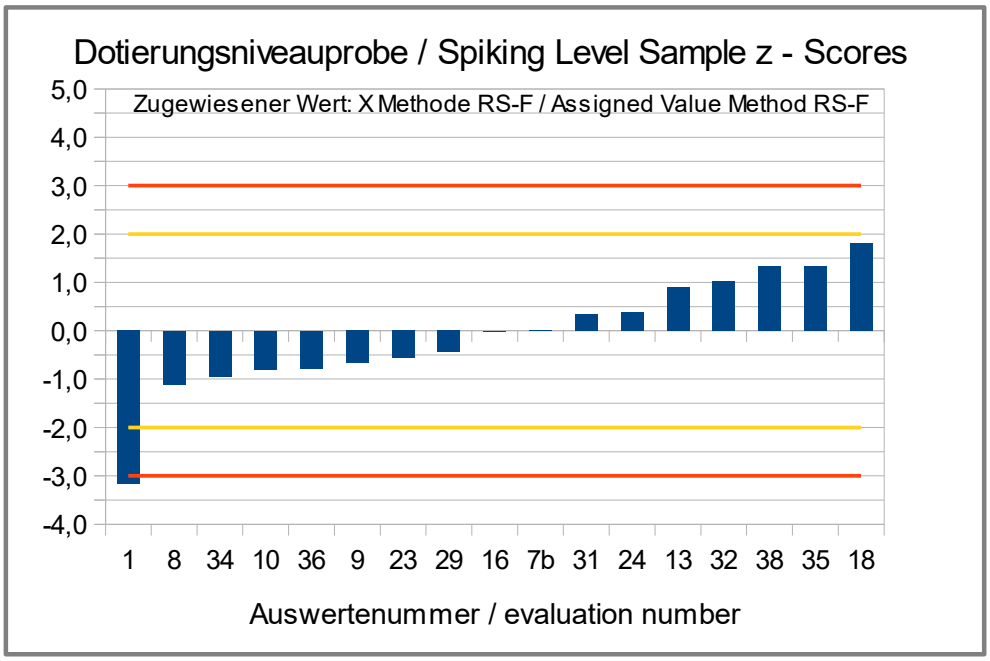


Abb./Fig. 24:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
12	**120**	299	8,0	25,4	**55**	-1,8	AQ	
37	33,5	**84**	-0,66	43,0	**92**	-0,30	AQ	
20	32,3	**81**	-0,78	36,9	**79**	-0,83	BC	
26	34,9	**87**	-0,52	42,7	**92**	-0,33	BC	
39	47,9	**119**	0,78	13,9	30	-2,8	BF	
7a	73,5	183	3,3	73,5	158	2,3	ES-I	result converted °
27	61,2	153	2,1	60,4	**130**	1,2	ES-I	result converted °
17				49,4	**106**	0,25	ES-II	result converted °
33	38,0	**95**	-0,21	41,0	**88**	-0,47	NL	
1	25,0	**62**	-1,5	21,0	45	-2,2	RS-F	
2	>20			>20			RS-F	
7b	120	299	8,0	110	237	5,5	RS-F	
8	86,7	216	4,6	93,6	201	4,1	RS-F	
9	100	249	6,0	138	297	7,9	RS-F	
10	96,0	239	5,6	135	290	7,6	RS-F	
13	147	366	11	190	408	12	RS-F	
15	>20			>20			RS-F	
16	120	298	7,9	121	259	6,4	RS-F	
18	174	434	13	170	366	11	RS-F	
22				>20			RS-F	
23	103	257	6,3	<2,5			RS-F	
24	131	328	9,1	151	324	9,0	RS-F	
29	107	266	6,6	125	269	6,8	RS-F	
31	130	324	9,0	130	280	7,2	RS-F	
32	151	377	11	179	385	11	RS-F	
34	91,7	229	5,1	144	309	8,4	RS-F	
35	160	399	12	157	338	9,5	RS-F	
36	96,5	241	5,6	135	289	7,6	RS-F	
38	160	399	12	127	274	7,0	RS-F	
11	22,0	**55**	-1,8	26,0	**56**	-1,8	SP	
14	42,0	**105**	0,19	47,0	**101**	0,04	SP	
30	34,0	**85**	-0,61	44,0	**95**	-0,22	SP	
6	204	508	16	201	432	13	VT	

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	9	Number in RA	10
Percent in RA	31	Percent in RA	34

* 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
ES-I = ELISA-Systems, new
ES-II = ELISA-Systems
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:
31% (9) 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 B 34% (10) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

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		
1	negative		positive		2/2 (100%)	ASU	
11	negative		positive		2/2 (100%)	ASU	
25	negative	<2.5	positive	9,40	2/2 (100%)	ASU	
4	negative		positive	10,0	2/2 (100%)	MS	
3	negative		positive		2/2 (100%)	SFA	
5	negative		positive		2/2 (100%)	SFA	
15	negative		positive		2/2 (100%)	SFA	
38	negative	<1	positive	176	2/2 (100%)	SFA-ID	
10	negative		positive		2/2 (100%)	div	
19	negative		positive		2/2 (100%)	div	
28	negative		positive		2/2 (100%)	div	
32	negative		positive		2/2 (100%)	div	
33a	negative		positive	13,0	2/2 (100%)	div	
33b	negative		positive	12,0	2/2 (100%)	div	
36	negative		positive		2/2 (100%)	div	

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

Methods:
ASU = ASU §64 Methode/method
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 B.

Quantitative Valuation PCR: Sample B

An evaluation of the quantitative results was not carried out because too few results were available (without considering result no. 38).

Characteristics: Quantitative evaluation ELISA Sesame (for information)

Sample B

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	
Mean	44,0
Median	12,0
Robust Mean (X_{pt})	**12,7**
Robust standard deviation (S*)	**4,33**
Target range:	
Target standard deviation σ_{pt}	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty U(X_{pt})	
Results in the target range	
Percent in the target range	

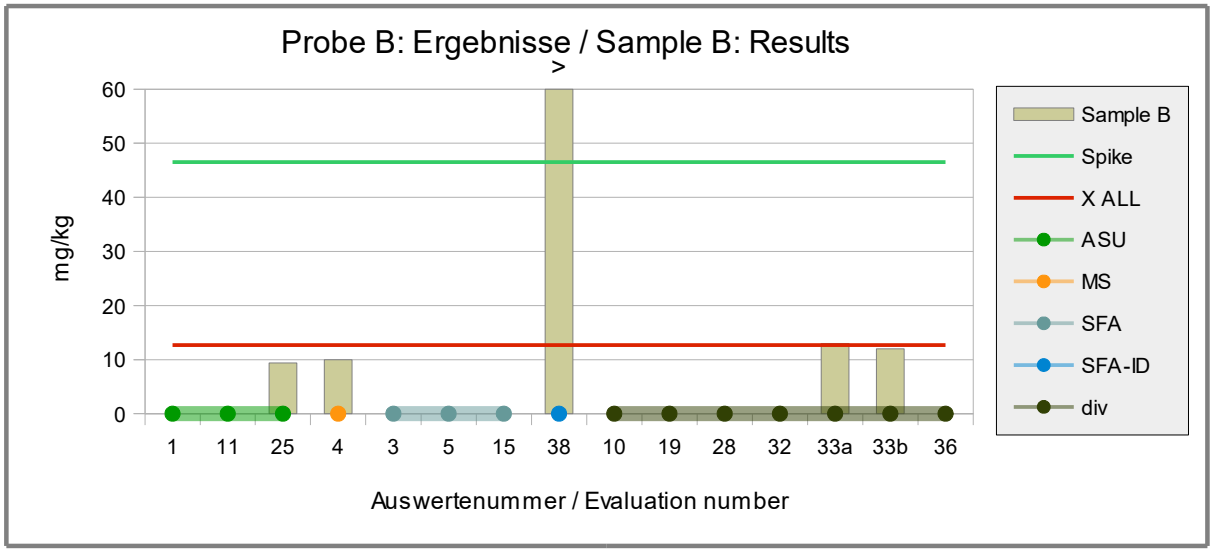


Abb./Fig. 25: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

An evaluation of the quantitative results was not carried out because there were only a few results with increased variability.

Evaluation number	Sesame	Sesame	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
1	positiv			ASU	
11	positiv			ASU	
25	positiv	15,0		ASU	
4	positiv	40,0		MS	
3	positiv			SFA	
5	positiv			SFA	
15	positiv			SFA	
38	positiv	72,4		SFA-ID	
10	positiv			div	
19	positiv			div	
28	positiv			div	
32	positiv			div	
33a	positiv	10,0		div	
33b	positiv	7,00		div	
36	positiv			div	

Number positive	15
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positiv

Methods:

ASU = ASU §64 Methode/method
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.

Characteristics: Quantitative evaluation ELISA Sesame (for information)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	
Mean	28,9
Robust Mean	28,9
Median	**15,0**
Robust standard deviation (S*)	**31,3**
Target range:	
Target standard deviation σ_{pt}	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty U(X_{pt})	
Results in the target range	
Percent in the target range	

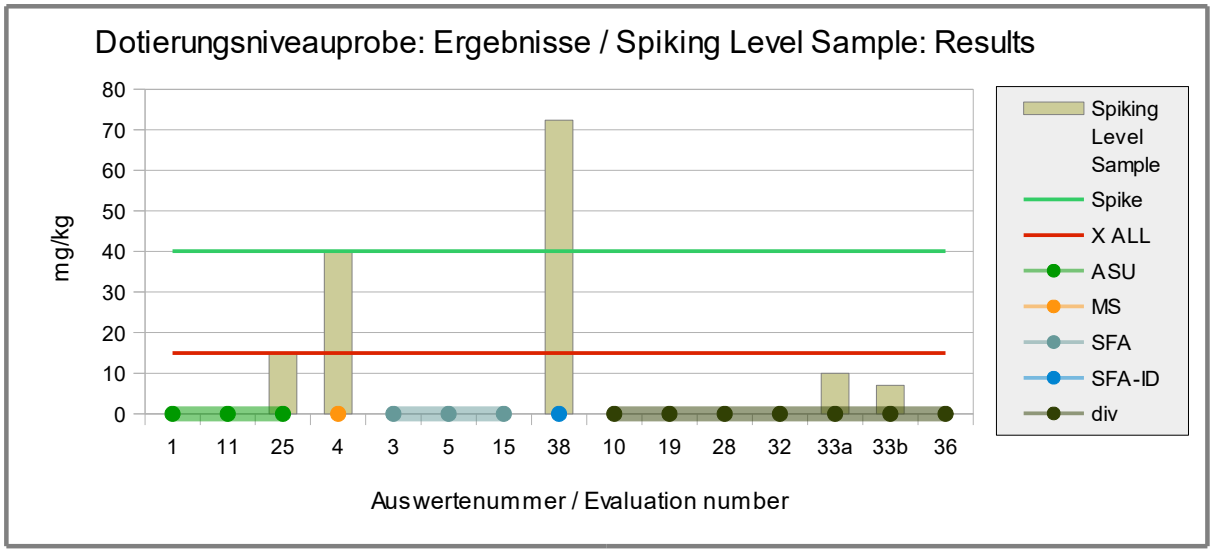


Abb./Fig. 26: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Sesame: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
1							ASU	
11							ASU	
25	15,0	37	-2,5	9,40	20	-3,2	ASU	
4	40,0	**100**	-0,01	10,0	22	-3,1	MS	
3							SFA	
5							SFA	
15							SFA	
38	72,4	181	3,2	176	378	11	SFA-ID	
10							div	
19							div	
28							div	
32							div	
33a	10,0	25	-3,0	13,0	28	-2,9	div	
33b	7,00	17	-3,3	12,0	26	-3,0	div	
36							div	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	0
Percent in RA	20	Percent in RA	0

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

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:

One out of 5 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 B none of the recovery rates were within the range of acceptance.

The related z-scores are based on the target standard deviation of 25%.

4.3 Participant z-Scores: overview table

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

Evaluation number	ELISA Mustard: Xpt (div. Methods)		ELISA Mustard: Xpt (Method: RS-F)		ELISA Mustard: Xpt (Method: VT)		ELISA Sesame: Xpt („Peak 42“ or „Peak 35“)		ELISA Sesame: Xpt („Peak 140“ or „Peak 123“)		ELISA Sesame: Xpt (Method: RS-F)	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample°	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
1	8,6	4,9	-	-	-	-	-	-	-3,4	-3,2	-3,4	-3,2
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	0,33	0,72	-	-	-0,09	1,4	-	-	1,7	2,6	-	-
7/7a	0,21	-0,90	-	-	-0,19	-0,48	3,1	4,4	-	-	-	-
7b	-	-	-	-	-	-	-	-	-0,86	-0,11	-0,78	0,00
8	-	-	-	-	-	-	-	-	-1,3	-1,2	-1,3	-1,1
9	-	-	-	-	-	-	-	-	-0,06	-0,76	0,04	-0,67
10	0,37	2,4	1,4	2,1	-	-	-	-	-0,16	-0,89	-0,06	-0,80
11/ 11a	-2,2	0,65	-	-	-	-	-1,5	-1,5	-	-	-	-
11b	1,7	0,07	-	-	1,1	0,62	-	-	-	-	-	-
12	1,4	0,31	-	-	-	-	-1,6	-1,8	-	-	-	-
13	-	-	-	-	-	-	-	-	1,4	0,76	1,6	0,90
14	-0,03	0,27	-	-	-	-	0,52	0,81	-	-	-	-
15	-0,60	-1,0	-	-	-	-	-	-	-	-	-	-
16	0,44	-0,54	-	-	0,01	-0,08	-	-	-0,56	-0,12	-0,47	-0,02
17	0,30	-	-	-	-0,12	-	0,75	-	-	-	-	-
18	0,45	-1,2	1,5	-0,51	-	-	-	-	0,85	1,6	0,97	1,8
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-0,45	-0,30	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-0,25	-	-	-	-	-	-	-	-0,66	-	-0,56
24	-1,4	-0,44	-0,85	0,04	-	-	-	-	0,29	0,26	0,40	0,38
25	0,42	0,28	1,4	0,56	-	-	-	-	-	-	-	-
26	-0,55	-0,94	-	-	-	-	0,11	0,00	-	-	-	-
27	-0,71	-1,3	-	-	-1,0	-0,97	1,8	3,0	-	-	-	-
28/ 28a	-	-	-	-	-	-	-	-	-	-	-	-
28b	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-0,43	-0,54	-0,34	-0,44
30	0,33	-0,61	-	-	-0,08	-0,15	0,23	-0,10	-	-	-	-
31	-	-	-	-	-	-	-	-	-0,29	0,22	-0,20	0,34
32	0,93	-0,61	-	-	0,46	-0,15	-	-	1,1	0,90	1,2	1,0
33/ 33a	-	-	-	-	-	-	-0,06	0,36	-	-	-	-
33b	-	-	-	-	-	-	-	-	-	-	-	-
34	22	13	-	-	-	-	-	-	0,10	-1,0	0,20	-0,94
35	-	-	-	-	-	-	-	-	0,48	1,2	0,59	1,3
36	-2,9	-3,1	-2,7	-1,9	-	-	-	-	-0,16	-0,87	-0,06	-0,78
37	-0,89	2,0	-	-	-	-	0,14	-0,16	-	-	-	-
38	-1,4	-0,88	-0,74	-0,29	-	-	-	-	-0,37	1,2	-0,27	1,3
39	-1,3	0,63	-	-	-	-	-2,7	1,5	-	-	-	-

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

° z'-Score

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Mustard:		ELISA Sesame:		PCR Celery:		PCR Mustard:		PCR Sesame:	
	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample	Sample B	Sp. Level Sample
1	13	13	-2,2	-1,5	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-0,15	-2,4	-2,1	-3,1	-0,01
5	-	-	-	-	-	-	-	-	-	-
6	1,9	5,2	13	16	-	-	-	-	-	-
7/ 7a	1,7	2,1	2,3	3,3	-	-	-	-	-	-
7b	-	-	5,5	8,0	-	-	-	-	-	-
8	-	-	4,1	4,6	-	-	-	-	-	-
9	-	-	7,9	6,0	-	-	-	-	-	-
10	1,9	8,6	7,6	5,6	-	-	-	-	-	-
11/ 11a	-1,6	5,1	-1,8	-1,8	-	-	-	-	-	-
11b	3,7	4,0	-	-	-	-	-	-	-	-
12	3,4	4,4	-1,8	-2,1	-	-	-	-	-	-
13	-	-	12	11	-	-	-	-	-	-
14	1,4	4,3	0,04	0,19	-	-	-	-	-	-
15	0,62	1,8	-	-	-	-	-	-	-	-
16	2,0	2,8	6,4	7,9	-	-	-	-	-	-
17	1,8	-	0,25	-	-	-	-	-	-	-
18	2,0	1,5	11	13	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-
20	-	-	-0,83	-0,78	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-
23	-	3,3	-	6,3	-	-	-	-	-	-
24	-0,52	3,0	9,0	9,1	-	-	-	-		
25	2,0	4,4	-	-	-	-	-	-	-3,2	-2,5
26	0,69	2,0	-0,33	-0,52	-	-	-	-	-	-
27	0,46	1,2	1,2	2,1	-	-	-	-	-	-
28/ 28a	-	-	-	-	-	-	-	-	-	-
28b	-	-	-	-	-	-	-	-	-	-
29	-	-	6,8	6,6	-	-	-	-	-	-
30	1,9	2,6	-0,22	-0,61	-	-	-	-	-	-
31	-	-	7,2	9,0	-	-	-	-	-	-
32	2,7	2,6	11	11	-	-	-	-	-	-
33/ 33a	-	-	-0,47	-0,21	-3,7	-3,7	-2,0	-2,7	-2,9	-3,0
33b	-	-	-	-	-3,2	-3,4	-2,0	-2,5	-3,0	-3,3
34	31	29	8,4	5,1	-	-	-	-	-	-
35	-	-	9,5	12	-	-	-	-	-	-
36	-2,5	-2,3	7,6	5,6	-	-	-	-	-	-
37	0,21	7,8	-0,30	-0,66	-	-	-	-	-	-
38	-0,41	2,1	7,0	12	0,58	2,0	5,5	-2,4	11	3,2
39	-0,34	5,1	-2,8	0,78	-	-	-	-	-	-

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):

-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)

-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)

-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 ELISA: Mustard

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	12	07.07.20	negative	<LOD	-	45,1	-	44,5	1	2		Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	37	25.06.20	negative	<2	positive	25,8	positive	62		2		Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
BC	26	02.07.20	negative	<2	positive	28,7	positive	31,6	2	2	50	Whole Mustard	BioCheck ELISA Mustard-Check
BF	39	21/8	negative	0	positive	22,4	-	47,8	0,13	1		Mustard	MonoTrace Mustard ELISA kit, BioFront Technologies
NL-E	23	15.06.	positive	38,9	negative	<2,00	positive	38,7	1	2	25	Mustard	NutriLinia; Mustard-E Kit
OS	15		negative	< 2	positive	28,28	positive	30,63		2		Mustard	ORSELL EZ-PLATE MUSTARD
RS-F	1		negative		positive	105	positive	92	0,5	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	2	29.06.20	negative	<0,5	positive	>13,5	positive	>13,5		0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	10	01.07.20	-	<0.5	-	36,3	-	66,4	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	13	06.07.20	negative	<2,5	positive	>13,5	positive	>13,5	-	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	18	02.07.20	negative		positive	37	positive	29	0,5	0,5	39,44	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	22	18.06.20	-	<0,5	-	>13,5	-			0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	24	1st July	negative	<0.5	positive	21,29	positive	36,74	0,5	0,5	30,94	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	25	12.08.20	-	<0.5	-	36,7	-	44,2				Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	36	19.08.20	-	<	-	8,9	-	8,9		0,5	22	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	38	09.07.20	negative	<0.5	positive	22,01	positive	32,18	0,5	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
SP	11a	25.6.	negative	<2	positive	15	positive	48	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins
SP	14	15.06.20	negative	0	positive	33	positive	44	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins
VT	6	06.07.20	negative	<1	positive	35,98	positive	48,73		1		Mustard	Veratox Mustard, Neogen
VT	7	2020/6/23	negative	<2.5	positive	35	positive	32		5		Mustard	Veratox Mustard, Neogen
VT	11b	18.06.	negative	<2,5	positive	47	positive	42	1,5	2,5		Mustard	Veratox Mustard, Neogen
VT	16	26.06.20	negative		positive	36,9	positive	35,7		2,5		Mustard	Veratox Mustard, Neogen
VT	17	21.07.20	negative	<1.0	positive	35,7	Not tested		1	2,5		Mustard	Veratox Mustard, Neogen
VT	27	24.06.20	negative	ND	positive	27,3	positive	27,6		2,5		Mustard	Veratox Mustard, Neogen
VT	30	20.07.20	negative	<2,5	positive	36	positive	35	2,5	2,5	50	Mustard	Veratox Mustard, Neogen
VT	32		negative		positive	41	positive	35		2,5		Mustard	Veratox Mustard, Neogen
VT	34	July/Aug	negative	<2.5	positive	65,7	positive	53	2,5	2,5	28,1	Mustardprotein	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. Abbr.	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	12	Polyclonal to whole Mustard	Extraction Buffer heated to 60 C. 1g sample extracted in 20 ml extraction buffer. Shake for 15 minutes and then centrifuge for 10 minutes.	yes	
AQ	37			yes	
BC	26		0.5g sample/10ml extraction buffer/15mis/60C	yes	
BF	39	Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	no	
NL-E	23	mustard proteins, o.A.	extraction buffer Mustard-E; 60°C, 5 min	yes	
OS	15			yes	
RS-F	1			yes	
RS-F	2				
RS-F	10			yes	
RS-F	13	-	Kit extraction solution / 10min / 60ºC	NO	
RS-F	18	Mustard protein (not specified by provider)	As per kit instructions	yes	
RS-F	22		mustard extraction buffer, 10 min, 60ºC	yes	
RS-F	24			yes	
RS-F	25				
RS-F	36			yes	
RS-F	38		As Per Kit Instructions	Yes	
SP	11a	detects mustard proteins	As Per Kit Instructions	yes	
SP	14				Additional dilution of 1:5 after extraction
VT	6		2 g of sample in 125 mL of diluited extraction buffer (1:10); 15 minutes, 60°C	NO	
VT	7	Poly/Mono	Tris EDTA Solution / 15 min / 60 C	yes	single result
VT	11b	detects mustard protein from seeds of white mustard (Sinapis alba), black mustard (Brassica nigra) and brown mustard (Brassica juncea)	As Per Kit Instructions	yes	
VT	16			Yes	
VT	17		Extraction: 60C pre-heated TRIS extraction buffer/ samples extracted in shaking waterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	Yes	
VT	27			yes	
VT	30		Tris/EDTA/55-60 degrees/15 min	yes	
VT	32			No	LFOP-TST-SOP-8828
VT	34		960ml dh20 + 40mls PBS Tween/30minutes/60°C - extraction. 30 minute ELISA analysis.	yes	

5.1.2 ELISA: Sesame

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	12	08.07.20	negative	<LOD	-	25,4	-	18,9	0,2	2		Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
AQ	37	23.06.20	negative	<2	positive	43	positive	33,5		2		Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
BC	20	02.07.20	-	<2	-	36,9	-	32,3	0,2	2	30	Sesame	BioCheck ELISA Sesame-Check
BC	26	02.07.20	negative	<2	positive	42,7	positive	34,9	2	2	50	Whole Sesame	BioCheck ELISA Sesame-Check
BF	39	21/8	negative	0	positive	13,9	-	47,9	0,3	1		Sesame	MonoTrace Sesame ELISA kit, BioFront Technologies
ES-I	7	2020/6/25	negative	<0.25	positive	18	positive	18		2,5		Sesameprotein	ELISA Systems Sesame ESSESE-48
ES-I	27	13 Aug.	negative	ND	positive	14,8	positive	15		0,25		Sesameprotein	Elisa Systems ESSESE-48
ES-II	17	02.07.20	negative	<0.125	positive	12,1	Not tested		0,125	0,25		Sesameprotein	ELISA Systems Sesame ESSESRD-48
NL	33	20.08.20	negative		positive	41	positive	38	0,2	2	30	Sesame	NutriLinia NC-6005
RS-F	1		negative		positive	21	positive	25	2,5	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	2	29.06.20	negative	<2,5	positive	>20	positive	>20		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	7	2020/6/25	negative	<2.5	positive	110	positive	120		25		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	8	24.06.20	-	<2,5	-	93,6	-	86,7	<0,14	<2,5	50	Sesame	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	9	08.07.20	negative		positive	138	positive	100	0,1	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	10	13.07.20	-	<2.4	-	134,8	-	96	0,14	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	13	24.06.20	negative	<2,5	positive	>20	positive	>20	-	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	15		negative	< 2,5	positive	>20	positive	>20		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	16	23.06.20	negative		positive	120,5	positive	119,5		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	18	06.08.20	negative		positive	170	positive	174	2,5	2,5	38,64	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	22	10.07.20	-	<2,5	-	>20	-			2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	23	16.06.	positive	100,6	negative	<2,5	positive	103,1	0,14	2,5	25	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	24	22nd July	negative	<2.5	positive	150,55	positive	131,38	2,5	2,5	27,13	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	29		negative	<2.5	positive	125	positive	106,7		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	31	10.08.20	-	<2.5	-	130	-	130	0,2	2,5	26	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	32		negative		positive	179	positive	151	1,2	4		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	34	July/Aug	negative	<2.5	positive	143,7	positive	91,7	2,5	2,5	49,4	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	35		negative	< 2,5	positive	157	positive	160	2,5	5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	36	19.08.20	-	<	-	134,5	-	96,5		2,5	24	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	38	09.07.20	negative	<2.5	positive	127,33	positive	159,92	2,5	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
SP	11	18.06.	negative	<2	positive	26	positive	22	1,5	2		Sesame	SensiSpec ELISA Sesame, Eurofins
SP	14	15.06.20	negative	0	positive	47	negative	42	0.2	2		Sesame	SensiSpec ELISA Sesame, Eurofins
SP	30	20.07.20	negative	<2,0	positive	44	positive	34	0,5	2	50	Sesame	Eurofins Technologies
VT	6	06.07.20	negative	<1	positive	200,79	positive	203,63		1		Sesame	Veratox Sesame Allergen, Neogen

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

Continuation ELISA Sesame:

Meth. Abbr.	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	12	Polyclonal to whole Sesame	Extraction Buffer heated to 60 C. 1g sample extracted in 20 ml extraction buffer. Shake for 15 minutes and then centrifuge for 10 minutes.	yes	
AQ	37			yes	
BC	20			yes	
BC	26		0.5g sample/10ml extraction buffer/15mis/60C	yes	
BF	39	Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	no	
ES-I	7	Polyclonal/ Monoclonal	Extraction solution concentrate / 15 mins / 60C	yes	single result
ES-I	27			yes	
ES-II	17	Anti-Sesame seed 2S-albumin	Extraction: Room temperature PBS extraction buffer (pH check) and samples extracted in shaking waterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	Yes	
NL	33		As Per Kit Instructions	yes	expanded MU (k=2)
RS-F	1			yes	
RS-F	2				
RS-F	7		Extraction solution concentrate / 15 mins / 60C	yes	single result
RS-F	8			NO	
RS-F	9	according to Testkit	As Per Kit Instructions	yes	
RS-F	10			yes	
RS-F	13	-	Kit extraction solution / 10min / 60ºC	YES	sample B: 189,8mg/Kg and Spiking level sample 146,8mg/Kg (Results out of accreditation scope)
RS-F	15			yes	
RS-F	16			Yes	
RS-F	18	Sesame protein (not specified by provider)	As per kit instructions	yes	
RS-F	22		Sesame extraction buffer, 10 min, 60ºC	no	
RS-F	23	Sesame proteins, o.A.	Extraktionspuffer Sesame 60°C, 10 min	yes	
RS-F	24			yes	
RS-F	29			NO	
RS-F	31	Sesame protein	AEP, 60C, 10mins, centrifuge 2500g	yes	
RS-F	32			yes	LFOP-TST-SOP-8867
RS-F	34		20mls Sesame extraction buffer (containing skim milk powder) - 10 minutes/60°C - extraction. 30 minute ELISA analysis.	yes	
RS-F	35			yes	
RS-F	36			yes	
RS-F	38		As Per Kit Instructions	Yes	
SP	11	detects sesame proteins	As Per Kit Instructions	yes	
SP	14				Additional dilution of 1:5 after extraction
SP	30		Tris/EDTA/60 degrees/15 min	yes	
VT	6		1 g of sample in 125 mL of diluited extraction buffer (1:10); 15 minutes, 60°C	YES	

5.1.3 PCR: Celery

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	1		negative		positive		positive		100			Celery-DNA	ASU §64 Methode/method
ASU	11	18.06.20	negative		positive		positive		50			Please select!	ASU §64 Methode/method
ASU	23	15.06.	positive	-	negative	-	positive	-	1		25	Celery-DNA	ASU §64 Methode/method
CEN	28	27.07.20	negative		positive		positive		5	nd		Please select!	CEN/TS 15634-2
CEN	32		negative		positive		positive		10			Celery-DNA	EN 15634-2:2019
IM	8	23.06.20	negative		positive		positive		0,4			Please select!	other: Imegen
MS	4	06.07.20	negative		negative		positive	30	10	10	45	Please select!	Auswahl PCR-Methoden
SFA	3	29.06.20	negative		positive		positive		0,4	1		Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative		positive		positive					Please select!	Selection PCR-Methods
SFA	15		negative		positive		positive		0,4			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	22	24.06.20	negative		positive		positive		2			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	34	Jun/Jul	negative		positive		positive		5			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	21	29.06.20	negative		positive		positive					Celery-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	38	24.07.20	negative	<1	positive	41,49	positive	46,8	1	1		Celery	Sure Food Allergen ID, R-Biopharm / Congen
div	10	06.07.20	negative		positive		positive					Celery-DNA	andere: bitte eingeben!
div	18	30.06.20	negative		positive		positive		1			Please select!	Selection PCR-Methods
div	19	01.08.20	negative		positive		positive		0,008				in-house method
div	24	7th August	negative	<1	positive	>1	positive	>1	1	1		Celery	other: please fill in!
div	33a	20.08.20	negative		positive	3	positive	2			40	Celery seed, dried	in house
div	33b	20.08.20	negative		positive	7	positive	5			40	Celery seed, dried	in house
div	36	16.07.20	negative		positive		positive		0,4			Celery-DNA	LifePrint: detection of Celery DNA
div	37	17.08.20	negative		positive		positive		10			Celery	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. Abbr.	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	1			yes	
ASU	11		CTAB, Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	§64 LFGB L 08.00-56:2014-08
ASU	23	MDH-Gen	CTAB; Proteinase K; Chloroform; Clean-up: Dneasy Mericon Food Kit (Qiagen)	yes	
CEN	28	Manitol déshydrogenase	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
CEN	32		Extraction by EN 15634-2:2019 (CTAB) / Realtime PCR/ 40 cycles	No	EN 15634-2:2019
IM	8			yes	
MS	4	Celery-DNA	Microsynth	yes	
SFA	3	Celery	CTAB Precipitaion, QIAgen PCR Purification Kit, Real Time PCR	no	
SFA	5		CTAB extraction + CONGEN PCR		
SFA	15			yes	
SFA	22		prep advance surefood/taq polymerase/ RT PCR/45 cycles	yes	
SFA	34		Extraction kit- Neogen Biokit DNA extraction Kit for GMO & Allergen.	yes	
SFA-4p	21		SureFood Prep Advanced Protokoll 1	no	
SFA-ID	38		As per Kit Instructions	Yes	
div	10	Mannitoldehydrogenase-Gen	in house RT-qPCR System	yes	
div	18	Celery DNA (not specified by provider)	As per kit instructions	no	
div	19			yes	
div	24	MTD	inhouse primers used	yes	
div	33a	Mannitol Dehydrogenase	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2)
div	33b	internal transcribed spacer	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2); celery multicopy
div	36		Extraction> Nucleo Spin Food, Real Time PCRQuantStudio5. 7500 Fast and CFX-96 deep well	yes	
div	37		gel electrophoresis	yes	

5.1.4 PCR: Mustard

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	11	18.06.20	negative		positive		positive		10			Please select!	ASU §64 Methode/method
ASU	23	15.06.	positive	-	negative	-	positive	-	4		25	Mustard-DNA	ASU §64 Methode/method
ASU	35		negative		positive		positive					Please select!	ASU §64 Methode/method
CEN	32		negative		positive		positive		10			Mustard-DNA	CEN/TS 15634-5:2016
GR	36	16.07.20	negative		positive		positive		0,4			Mustard-DNA	SPECIALfinder Assay, real time PCR, Generon
MS	4	06.07.20	negative		positive	10	positive	10	10	10	45	Please select!	Auswahl PCR-Methoden
SFA	3	25.06.20	negative		positive		positive		0,4	1		Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative		positive		positive					Please select!	Selection PCR-Methods
SFA	8	23.06.20	negative		positive		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15		negative		positive		positive		0,4			Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	38	24.07.20	negative	<1	positive	58,02	positive	8,59	1	1		Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-4p	21	29.06.20	negative		positive		positive					Mustard-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
div	10	07.07.20	negative		positive		positive					Mustard-DNA	
div	19	01.08.20	positive		positive		negative		0,008				in-house method
div	28a	27.07.20	negative		positive		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	28b	27.07.20	negative		negative		negative		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	33a	20.08.20	negative		positive	12	positive	7			40	Mustard	in house
div	33b	20.08.20	negative		positive	12	positive	8			40	Mustard	in house

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation PCR Mustard:

Meth. Abbr.	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	11		CTAB, Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	§64 LFGB L 08.00-65:2017-10
ASU	23	MADS-D-Gene	CTAB; Proteinase K; Chloroform; Clean-up: Dneasy Mericon Food Kit (Qiagen)	yes	
ASU	35		mod.	yes	
CEN	32		Extraction by PD CEN/TS 15634-5:2016 (CTAB) / Realtime PCR/ 40 cycles	No	CEN/TS 15634-5:2016
GR	36		Extraction> Nucleo Spin Food, Real Time PCRQuantStudio5. 7500 Fast and CFX-96 deep well	no	
MS	4	Mustard-DNA	Microsynth	yes	
SFA	3	Mustard	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
SFA	5		CTAB extraction + CONGEN PCR		
SFA	8			yes	
SFA	15			yes	
SFA	38		As per Kit Instructions	Yes	
SFA-4p	21		SureFood Prep Advanced Protokoll 1	no	
div	10	SinA1-Gene	in house RT-qPCR System	yes	
div	19			yes	
div	28a	MADS-D	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	28b	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	33a	MADS-D protein, Gypsy-like retro element	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2)
div	33b	internal transcribed spacer, Gypsy-like retro element	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2); mustard multicopy

5.1.5 PCR: Sesame

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	1		negative		positive		positive		0,04			Sesame-DNA	ASU §64 Methode/method
ASU	11	18.06.20	negative		positive		positive		10			Please select!	ASU §64 Methode/method
ASU	25	12.08.20	-	<2.5	-	9,4	-	15				Sesame	ASU §64 Methode/method
MS	4	06.07.20	negative		positive	10	positive	40	10	10	44	Please select!	
SFA	3	26.06.20	negative		positive		positive		0,4	1		Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative		positive		positive					Please select!	Selection PCR-Methods
SFA	15		negative		positive		positive		0,4			Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	38	13.07.20	negative	<1	positive	175,73	positive	72,39	1	1		Sesame	Sure Food Allergen ID, R-Biopharm / Congen
div	10	06.07.20	negative		positive		positive					Sesame-DNA	
div	19	01.08.20	negative		positive		positive		0,008				in-house method
div	28	27.07.20	negative		positive		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 allergenic food ingredients Sesame, almond, lupine and Brazil nur - J. Verbr. Lebensm. - DOI 10,1007/s00003-014-0868-x
div	32		negative		positive		positive		10			Sesame-DNA	LFOD-TST-SOP-8852
div	33	20.08.20	negative		positive	13	positive	10			40	Sesame	in house
div	33	20.08.20	negative		positive	12	positive	7			40	Sesame	in house
div	36	16.07.20	negative		positive		positive		0,4			Sesame-DNA	LifePrint: detection of Sesame DNA

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abbr.	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	1			yes	
ASU	11		CTAB, Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	§64 LFGB L 08.00-19:2014-08
ASU	25				
MS	4	Sesame-DNA	Microsynth	yes	
SFA	3	Sesame	CTAB Präzipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
SFA	5		CTAB extraction + CONGEN PCR		
SFA	15			yes	
SFA-ID	38		As per Kit Instructions	No	
div	10	Oleosin-Gene	in house RT-qPCR System	yes	
div	19			yes	
div	28	Albumine 2S	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	32		Extraction by ISO 21571:2005 (CTAB) / Realtime PCR/ 40 cycles	Yes	LFOD-TST-SOP-8852
div	33a	2s Albumin	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2)
div	33b	internal transcribed spacer	NucleoSpin Food Kit , Real Time PCR 45 Cycles	yes	expanded MU (k=2); sesame multicopy
div	36		Extraction> Nucleo Spin Food, Real Time PCRQuantStudio5. 7500 Fast and CFX-96 deep well	no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL04 Sample B

Weight whole sample	3,65	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	25,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	61	24,3
2	5,04	61	24,2
3	5,04	56	22,2
4	5,00	48	19,2
5	5,00	52	20,8
6	5,02	49	19,5
7	5,06	56	22,1
8	5,04	53	21,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,5	Particles
Standard deviation	4,80	Particles
χ^2 (CHI-Quadrat)	2,96	
Probability	**89**	%
Recovery rate	86	%

Normal distribution		
Number of samples	8	
Mean	21,7	mg/kg
Standard deviation	1,91	mg/kg
rel. Standard deviaton	8,8	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,87**	
Recovery rate	86	%

Microtracer Homogeneity Test
DLA ptAL04 Spiking Level Sample

Weight whole sample	1,52	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	54	21,5
2	5,03	47	18,7
3	5,03	48	19,1
4	5,00	48	19,2
5	4,99	53	21,2
6	4,97	47	18,9
7	5,01	50	20,0
8	5,00	44	17,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	48,9	Particles
Standard deviation	3,28	Particles
χ^2 (CHI-Quadrat)	1,54	
Probability	**98**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	19,5	mg/kg
Standard deviation	1,31	mg/kg
rel. Standard deviaton	6,7	%
Horwitz standard deviation	10,2	%
HorRat-value	**0,66**	
Recovery rate	97	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL04 - 2020***
PT name	***Allergens IV: Celery, Mustard and Sesame in Instant Soup Powder***
Sample matrix (processing)	***Samples A + B:*** *Tomato cream soup (powder) / ingredients: tomato powder, potato flour, starch, sugar, salt, onion powder, rice flour, yeast extract, corn oil, malto-dextrin, spices (garlic, pepper), beetroot juice powder / beetroot juice powder, herbs (bay leaves, oregano), flavors, other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A, B + Spiking Level Sample:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Celery, Mustard and Sesame (Protein, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest August 21st 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		CZECH REPUBLIC
		GREAT BRITAIN
		SPAIN
		USA
		SWITZERLAND
		Germany
		CANADA
		ITALY
		Germany
		SPAIN
		Germany
		FRANCE
		SWEDEN
		Germany
		ISRAEL
		SPAIN
		SWITZERLAND
		SWITZERLAND
		FRANCE
		ITALY
		Germany
		Germany
		Germany
		CANADA
		CANADA
		ITALY
		GREAT BRITAIN
		SCOTLAND, UK
		FRANCE
		GREAT BRITAIN
		GREAT BRITAIN
		USA
		VIETNAM
		SPAIN
		Germany
		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

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked im-

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