Evaluation Report
proficiency test

DLA ptAL04 (2021)

Allergens IV:

Celery, Mustard and Sesame

in Mayonnaise

DLA - Proficiency Tests GmbH
Hauptstr. 80
23845 Oering/Germany

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

Coordinator of this PT:
Matthias Besler-Scharf, Ph.D.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Hauptstr. 80, 23845 Oering, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.
Akkreditierung *Accreditation* ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-EP-21534-01-00	nach / according to: ISO/IEC 17.043-2010 Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen Conformity Assessment - General Requirements for Proficiency Testing Die Akkreditierung gilt für den in der Urkundenanlage genannten Umfang. The accreditation is valid for the scope of the annex to the certificate of accreditation

Contents

1. Introduction

The participation in proficiency 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 mayonnaise. The basic composition of both sample A and sample B was the same (see table 1).
After homogenization of the basic mixture 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 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 into PE containers with srew cap, 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**
Mayonnaise Ingredients: 50% rapeseed oil, water, brandy vinegar, sugar, egg yolk, wheat starch, salt, modified starch, thickener: xanthan gum, guar gum, sodium alginate, acidity regulator: sodium acetate, natural flavor Nutrients per 100 g: Fat 51 g, Carbohydrates 7,0 g, Protein 0,5 g, Salt 2,1 g	100 g/100 g	99,7 g/100g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,8 g/100 g
Celery seed, ground - as celery* - thereof 20,0% total protein**	-	 47,8 mg/kg 9,55 mg/kg	 37,2 mg/kg 7,45 mg/kg
Mustard, yellow (Sinapis alba) ground, mixture of 9 products (Europe, Asia) - as mustard* - thereof 30,7% total protein**	-	 50,1 mg/kg 15,4 mg/kg	 48,9 mg/kg 15,0 mg/kg
Sesame, white (Sesamum indicum) ground, mixture of 10 products (Africa, Asia, South America) - as Sesame seed* - *thereof 24,5% total protein***	-	 47,5 mg/kg 11,6 mg/kg	 33,9 mg/kg 8,30 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	<0,3 g/100 g	<0,3 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].
Because pasty samples can not be analysed by the microtracer method, only the spiking level sample was measured. The microtracer analysis of the present PT showed a probability of 78%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value of 1,1. The HorRat value of >1,3 was accepted, because the probability was sufficient proof of homogeneity. 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, Veratox 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].

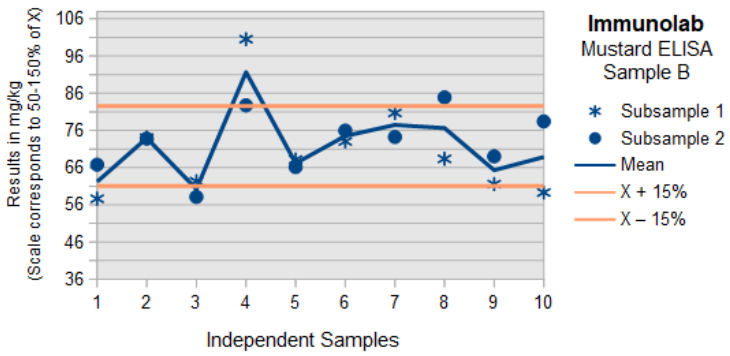
ELISA-Tests: Homogenität Senf / Homogeneity Mustard

Immunolab Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 71,8 ± 8,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	57,6	66,8	62,2
2	74,1	73,8	73,9
3	62,3	58,1	60,2
4	100,6	82,7	91,6
5	68,2	66,1	67,2
6	73,0	76,0	74,5
7	80,7	74,2	77,5
8	68,4	84,9	76,6
9	61,6	69,0	65,3
10	59,3	78,4	68,9

General average X	71,8	
SD of sample means Sx	9,18	12,8%
SD within-samples Sw	5,40	7,5%
SD between-samples Ss	8,35	11,6%

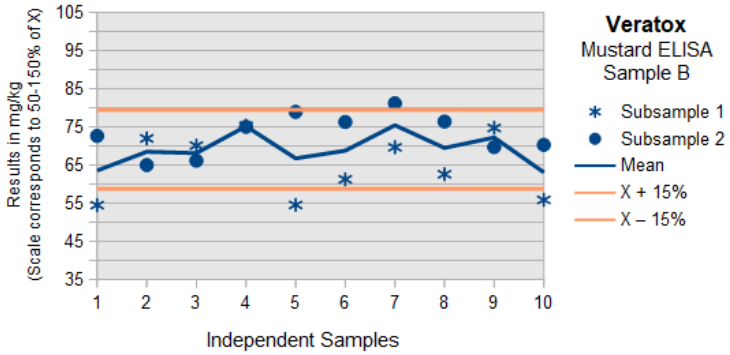


Veratox Mustard ELISA

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Mustard 69,1 ± 4,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	54,5	72,6	63,5
2	72,0	65,0	68,5
3	70,1	66,1	68,1
4	75,3	75,0	75,1
5	54,5	78,9	66,7
6	61,2	76,3	68,7
7	69,6	81,2	75,4
8	62,5	76,4	69,5
9	74,7	69,7	72,2
10	55,8	70,3	63,0

General average X	69,1	
SD of sample means Sx	4,22	6,1%
SD within-samples Sw	6,65	9,6%
SD between-samples Ss	< 4,22	< 6,1%

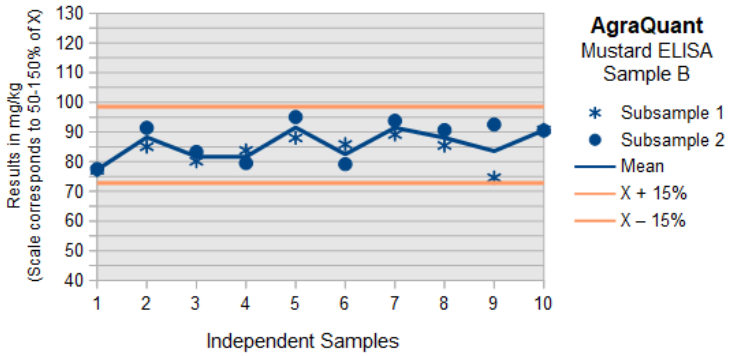


AgraQuant Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 85,7 ± 4,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	77,1	77,5	77,3
2	85,1	91,4	88,2
3	80,1	83,3	81,7
4	83,8	79,5	81,7
5	88,0	95,0	91,5
6	85,8	79,2	82,5
7	89,1	93,8	91,5
8	85,5	90,7	88,1
9	74,6	92,5	83,6
10	90,7	90,5	90,6

General average X	85,7	
SD of sample means Sx	4,95	5,8%
SD within-samples Sw	3,65	4,3%
SD between-samples Ss	4,22	4,9%



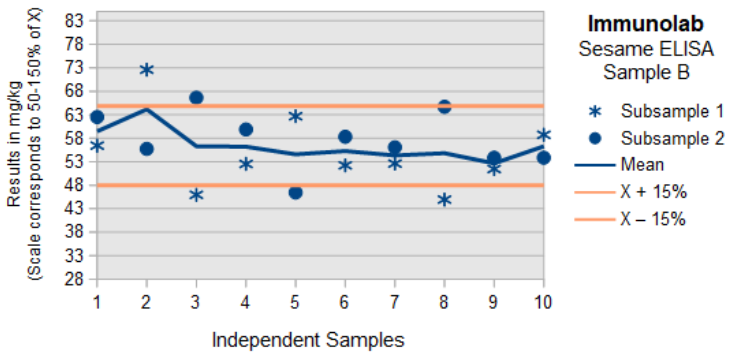
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Immunolab Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 56,4 ± 3,3 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	56,4	62,5	59,5
2	72,6	55,7	64,2
3	46,0	66,6	56,3
4	52,5	59,9	56,2
5	62,7	46,5	54,6
6	52,2	58,3	55,3
7	52,6	56,1	54,3
8	44,9	64,7	54,8
9	51,5	53,9	52,7
10	58,7	53,9	56,3

General average X	56,4	
SD of sample means Sx	3,25	5,8%
SD within-samples Sw	6,19	11,0%
SD between-samples Ss	<3,25	<5,8%

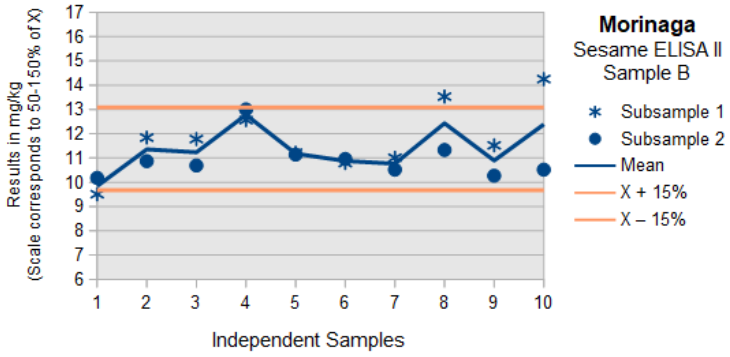


Morinaga Sesame ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame protein 11,4 ± 0,73 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	9,51	10,2	9,85
2	11,8	10,9	11,4
3	11,8	10,7	11,2
4	12,6	13,0	12,8
5	11,2	11,1	11,2
6	10,8	11,0	10,9
7	11,0	10,5	10,8
8	13,5	11,3	12,4
9	11,5	10,3	10,9
10	14,3	10,5	12,4

General average X	11,4	
SD of sample means Sx	0,904	7,9%
SD within-samples Sw	0,764	6,7%
SD between-samples Ss	0,725	6,4%

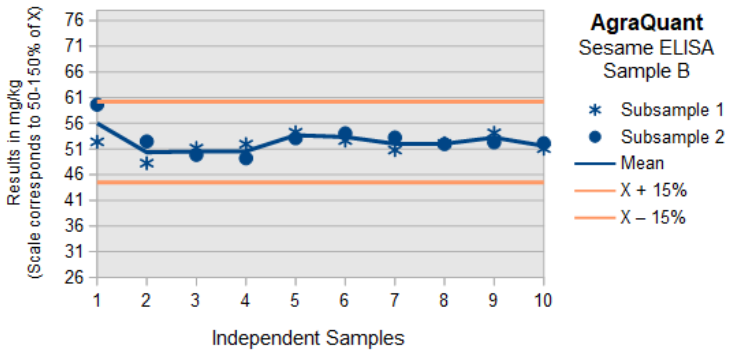


AgraQuant Sesame ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 52,3 ± 1,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	52,4	59,6	56,0
2	48,2	52,5	50,4
3	51,2	49,9	50,5
4	51,9	49,2	50,6
5	54,3	53,1	53,7
6	52,6	54,0	53,3
7	50,8	53,2	52,0
8	52,1	51,9	52,0
9	54,1	52,3	53,2
10	51,1	52,1	51,6

General average X	52,3	
SD of sample means Sx	1,77	3,4%
SD within-samples Sw	1,53	2,9%
SD between-samples Ss	1,40	2,7%



2.1.2 Stability

The food matrix of the sample material is mayonnaise, which is known to be stable for several months due to heat treatment and the low pH value. The storage stability and durability of the samples (microbial spoilage) was thus ensured 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. 0,32 (22,4°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 21st week of 2021. The testing method was optional. The tests should be finished at 23rd July 2021 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters celery, mustard and sesame in the range of mg/kg in the matrix of mayonnaise. 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.*

Note: Please cool samples on arrival (2 – 10 ° C)

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.

29 out of 30 participants submitted at least one result. One participant submitted no results.

3. Evaluation

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

For quantitative results of the spiking 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 15 – 43% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

The Working Group on Prolamin Analysis and Toxicity (WGPAT) coordinated a collaborative study with two commercial ELISA test kits for the determination of gluten using the monoclonal R5 antibody [24]. 12 food samples with gliadin in the range of 0 - 168 mg/kg were analyzed by 20 laboratories. Recovery rates ranged between 65 and 110%, relative repeatability deviations ranged from 13 - 25% (method 1) and 11 - 22% (method 2) while the relative reproducibility standard deviations ranged from 23 - 47% (method 1) and 25 - 33% (method 2). According to the authors both ELISA test kits fulfilled therefore the current validation criteria for ELISA methods [24].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [27]. The mean values for two matrices were in the concentration range of 0,3 - 16,1 mg/kg and 1,2 - 20,4 mg/kg, respectively. The lowest relative reproducibility standard deviations of the five test kits were for dark chocolate in the range of 20 - 42% and for cookies in the range of 23 – 61%.

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-36]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Celery seed	Sausage, cooked (100°C, 60 min)	98,1 45,5	98,1 % 114 %	- -	12,6% 27,9%	20,7% 34,7%	18,7% 28,5%	rt-PCR ASU 08.00-65
Celery seed	Sausage, autoclaved	10,5	10,5 %	-	25,8%	39,4%	34,9%	rt-PCR ASU 08.00-65
Mustard, brown / black	Sausage, autoclaved	146,7 50,0 15,8	147 % 125 % 158 %	-	12,3% 17,2% 15,4%	22,0% 31,6% 27,1%	20,2% 29,2% 24,8%	rt-PCR ASU 08.00-64
Mustard, brown / black	Sausage, autoclaved	168,3 52,9 17,6	168 % 132 % 176 %	-	11,4% 10,0% 23,1%	31,6% 23,1% 46,3%	29,5% 21,9% 43,3%	rt-PCR ASU 08.00-65
Mustard, white	Sausage, cooked (100°C, 60 min)	79,9 37,0 18,0 8,0	80 % 93 % 90 % 80 %	-	13,6% 15,7% 14,4% 15,4%	23,6% 29,2% 30,6% 26,1%	21,6% 27,0% 28,9% 23,7%	rt-PCR ASU 08.00-59
Mustard, white	Sausage, cooked (100°C, 60 min)	103,3 45,9	103 % 115 %	- -	11,8% 14,7%	17,1% 21,8%	14,9% 19,2%	rt-PCR ASU 08.00-65
Mustard, white	Sausage, autoclaved	11,7	11,7 %	-	24,1%	34,3%	29,8%	rt-PCR ASU 08.00-65
Sesame	Rice cookie	94,6 15,7 9,8	95 % 79 % 98 %	-	22,5% 26,0% 20,9%	27,5% 39,5% 33,5%	22,4% 35,0% 30,0%	rt-PCR ASU 18.00-19
Sesame	Wheat cookie Sauce powder	96,9 59,8	79 % 60 %	-	21,8% 22,2%	33,0% 43,2%	29,2% 40,2%	rt-PCR ASU 18.00-19
Sesame	Rice cookie	88,9 17,8 9,8	89 % 89 % 98 %	-	18,2% 34,2% 26,2%	30,5% 37,8% 37,0%	27,7% 29,1% 32,0%	rt-PCR ASU 18.00-22
Sesame	Wheat cookie Sauce powder	115 58,5	93 % 59 %	-	16,7% 30,8%	41,1% 44,4%	39,4% 38,7%	rt-PCR ASU 18.00-22

3.4.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].
Criteria for the level of performance of analytical methods for the quantitative determination of allergens in foods were recently elaborated e.g. by the Ministry of Health and Welfare (MHLW) in Japan [22], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [19-21], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [23] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [18].

Some of the relevant ELISA and PCR validation criteria of the mentioned panels are listed in tables 3 and 4, respectively.

Table 3: ELISA-Validation

Literature [18-24]	**Recovery rate**	**Repeatability standard deviation**	**Reproducibility standard deviation**
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% (a)	19,5 - 57,2% (a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

(a) = Example from an hypothetical proficiency scheme in the range of 0,5 - 5 mg/kg

Table 4: PCR-Validation

Literature [18]	**Recovery rate**	**Repeatability standard deviation**	**Reproducibility standard deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Based on the currently achievable level of performance of ELISA and PCR methods for the quantitative determination of allergens in foods, which could be deduced from the data of precision experiments and from validation criteria, we set a relative target standard deviation σ_{pt} of 25%.
This target standard deviation was applied for the statistical evaluation of the results by z-score or if necessary by z´-Score and was used for all assigned values mentioned in 3.1.

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

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

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

For information the z-scores below are calculated with a target standard deviation of 25%:

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/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 allergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.
The corresponding z-scores were calculated according to 3.5 with the target standard deviation of 25% (see 3.4.3).

4. Results

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

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

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

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

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

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

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

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

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

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

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

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

4.1 Proficiency Test 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		
8	negative		positive		2/2 (100%)	ASU	
9	negative		positive		2/2 (100%)	ASU	
1	negative		positive		2/2 (100%)	CEN	
20	negative		positive		2/2 (100%)	CEN	
10	negative		positive	0,16	2/2 (100%)	FP	
15	negative	<0,1	positive	<0,8	2/2 (100%)	FP	
17	negative	< 0,080	positive	0,58	2/2 (100%)	FP	
3	negative		positive		2/2 (100%)	IM	
7	negative		positive		2/2 (100%)	SFA	
16	negative		positive		2/2 (100%)	SFA	
21	negative	-	positive	-	2/2 (100%)	SFA	
18a	negative		positive		2/2 (100%)	SFA	
4	negative		positive		2/2 (100%)	SFA-4p	
12	negative	< 0,4	positive		2/2 (100%)	SFA-ID	
27	negative	<1	positive	25,4	2/2 (100%)	SFA-ID	
18b	negative		negative		1/2 (50%)	div	no positive sample detected
22	positive		negative		0/2 (0%)	div	samples interchanged?
25	negative		positive		2/2 (100%)	div	
26	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	1		17	
Number negative	18		2	
Percent positive	5		89	
Percent negative	95		11	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
FP = foodproof Detection Kit, BIOTECON Diagnostics
IM = Imegen
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.

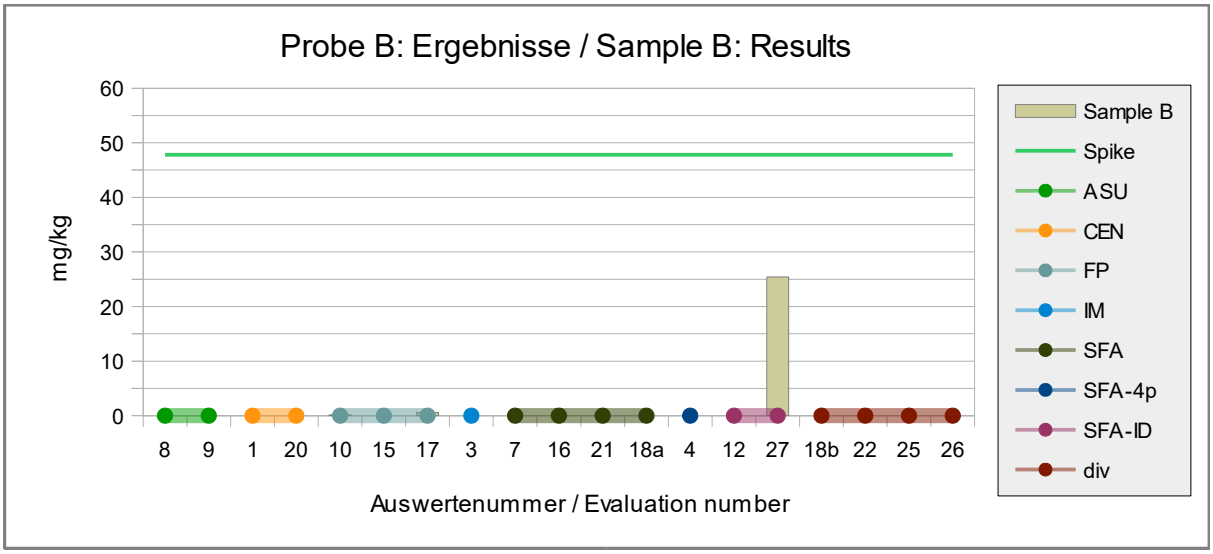


Abb./Fig. 1: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

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]			
8	positive			ASU	
9	positive			ASU	
1	positive			CEN	
20	positive			CEN	
10	positive	0,82		FP	
15	positive	<0,8		FP	
17	positive	3,1		FP	
3	positive			IM	
7	positive			SFA	
16	positive			SFA	
21	positive	-		SFA	
18a	positive			SFA	
4	positive			SFA-4p	
12	positive			SFA-ID	
27	positive	20,5		SFA-ID	
18b	negative			div	
22	positive			div	
25	positive			div	
26	positive			div	

Number positive	18	
Number negative	1	
Percent positive	95	
Percent negative	5	
Consensus value	positive	

Methods:

ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
FP = foodproof Detection Kit, BIOTECON Diagnostics
IM = Imegen
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:
95% positive results were obtained for the spiking level sample.

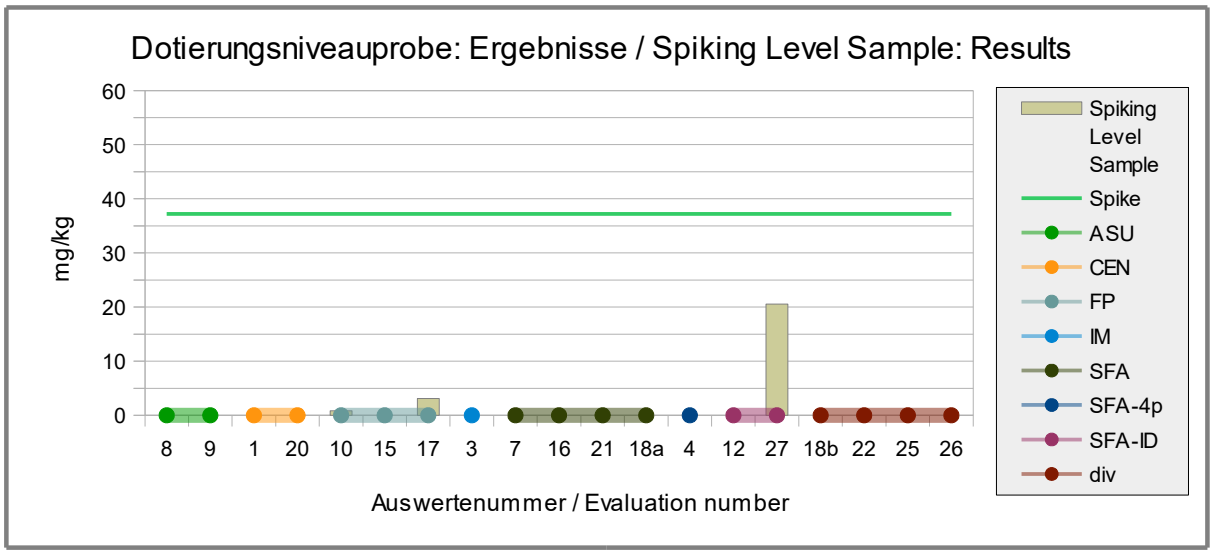


Abb./Fig. 2: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample B	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
8							ASU	
9							ASU	
1							CEN	
20							CEN	
10	0,82	2,2	-3,9	0,16	0,3	-4,0	FP	
15	<0,8			<0,8			FP	
17	3,1	8,3	-3,7	0,58	1,2	-4,0	FP	
3							IM	
7							SFA	
16							SFA	
21	-			-			SFA	
18a							SFA	
4							SFA-4p	
12							SFA-ID	
27	20,5	**55**	-1,8	25,4	**53**	-1,9	SFA-ID	
18b							div	
22							div	
25							div	
26							div	

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

* Wiederfindungsrate 100% Bezugsgröße: Selleriesamen, s. Seite 5

** Akzeptanzbereich der AOAC für Allergen-ELISAs

* 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 = European Committee for Standardization Method

FP = foodproof Detection Kit, BIOTECON Diagnostics

IM = Imegen

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 1 of 3 participants obtained with the spiking level sample as well as with the spiked food matrix sample B a recovery rate within the range of the AOAC-recommendation of 50-150%.

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	Probe A	Probe A	Probe B	Probe B	Qualitative Bewertung	Methode	Hinweis
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Übereinstimmungen mit Konsenswerten		
15	negative	<2	positive	71,3	2/2 (100%)	AQ	
13	negative	<2	positive	51,4	2/2 (100%)	BC	
23	negative	<2	positive	40,7	2/2 (100%)	BC	
17	negative	< 6,5	positive	39,1	2/2 (100%)	IL	result converted °
12	negative	<2	positive	28,7	2/2 (100%)	OS	
2	negative	<0,5	positive	>13,5	2/2 (100%)	RS-F	
8	negative	< 0,5	positive	11,4	2/2 (100%)	RS-F	
10	negative	<LOQ	positive	20,4	2/2 (100%)	RS-F	
16	negative	<0,5	positive	>13,5	2/2 (100%)	RS-F	
18	negative	<0.5	positive	81,2	2/2 (100%)	RS-F	
19	negative	<0.50	positive	49,9	2/2 (100%)	RS-F	
20	negative		positive	67,1	2/2 (100%)	RS-F	
21	negative	< 1,6	positive	167	2/2 (100%)	RS-F	result converted °
27	negative	<0.5	positive	79,2	2/2 (100%)	RS-F	
5	negative	<2.0	positive	34,9	2/2 (100%)	SP	
9	negative	<2	positive	73,0	2/2 (100%)	SP	
29	negative	<1	positive	95,0	2/2 (100%)	SP	
6	negative	<2.5	positive	76,9	2/2 (100%)	VT	
11	negative	<1.0	positive	66,1	2/2 (100%)	VT	
14	negative	< 2.5	positive	65,0	2/2 (100%)	VT	
24	negative	<2.5	positive	75,0	2/2 (100%)	VT	
26	negative	<2.5	positive	74,0	2/2 (100%)	VT	

° calculation p. 20

	Sample A		Sample B	
Number positive	0		22	
Number negative	22		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
IL = Immunolab
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	Senf	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Methode	Hinweis
	[mg/kg]					
15	71,3	0,71			AQ	
13	51,4	-0,60			BC	
23	40,7	-1,3			BC	
17	39,1	-1,4			IL	result converted °
12	28,7	-2,1			OS	
2	>13,5				RS-F	
8	11,4	-3,2	-3,3		RS-F	
10	20,4	-2,7	-2,7		RS-F	
16	>13,5				RS-F	
18	81,2	1,4	1,1		RS-F	
19	49,9	-0,70	-0,84		RS-F	
20	67,1	0,44	0,25		RS-F	
21	167	7,0	6,6		RS-F	result converted °
27	79,2	1,2	1,0		RS-F	
5	34,9	-1,7			SP	
9	73,0	0,83			SP	
29	95,0	2,3			SP	
6	76,9	1,1		0,31	VT	
11	66,1	0,37		-0,30	VT	
14	65,0	0,30		-0,36	VT	
24	75,0	0,96		0,20	VT	
26	74,0	0,89		0,15	VT	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
IL = Immunolab
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

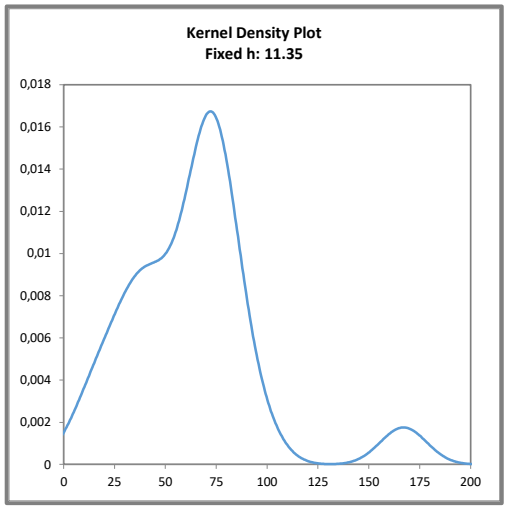


Abb. / Fig. 3:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a main peak with a shoulder and a small side peak.

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	20	7	5
Number of outliers	-	0	0
Mean	63,4	68,0	71,4
Median	66,6	67,1	74,0
Robust Mean (X_{pt})	**60,5**	**63,1**	**71,4**
Robust standard deviation (S*)	**26,7**	**46,6**	**6,20**
Target range:			
Target standard deviation σ_{pt}	**15,1**	**15,8**	**17,9**
lower limit of target range	**30,3**	**31,6**	**35,7**
upper limit of target range	**90,8**	**94,7**	**107**
Quotient S/σ_{pt}*	*1,8*	*3,0*	*0,35*
Standard uncertainty $U_{(Xpt)}$	*7,45*	*22,0*	*3,50*
Results in the target range	*15*	*4*	*5*
Percent in the target range	*75*	*57*	*100*

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

Comments to the statistical characteristics and assigned values:

The evaluations of all methods and method VT showed a normal to low variability of results, with quotients S/σ_{pt} below 2,0 and 1,0, respectively. 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 evaluation of the results of method RS-F showed an increased variability of the results. The quotient S/σ_{pt} was well above 2.0. evaluation was carried out informatively. An evaluation using the z'-score taking into account the standard uncertainty was dispensed with, since this would make the target range unsuitably large.*

The robust means of the evaluations were 121%, 126% and 143% 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.36).

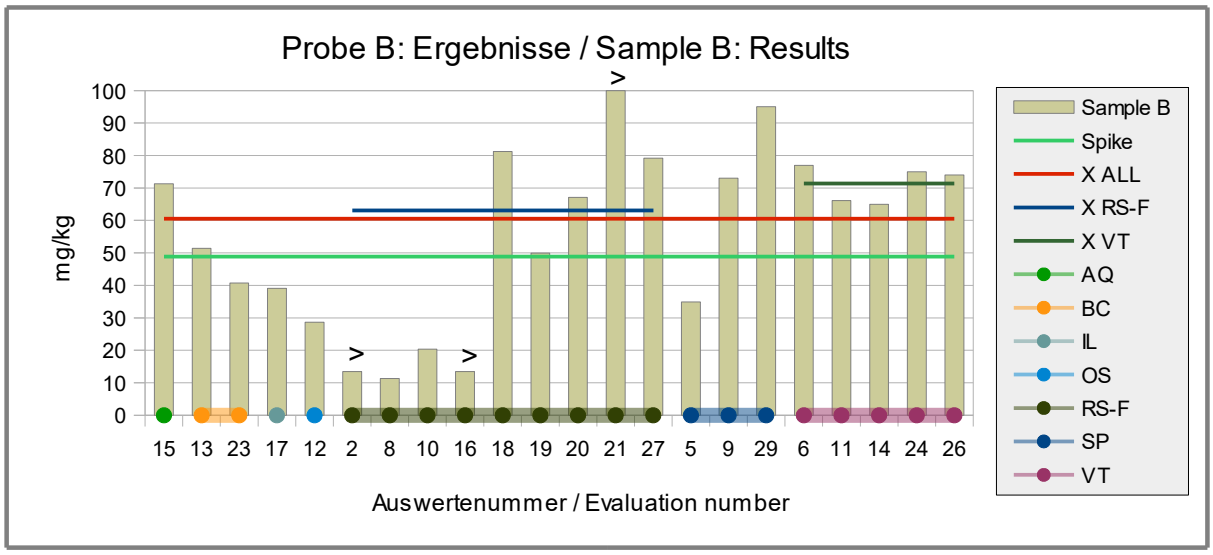


Abb./Fig. 4: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

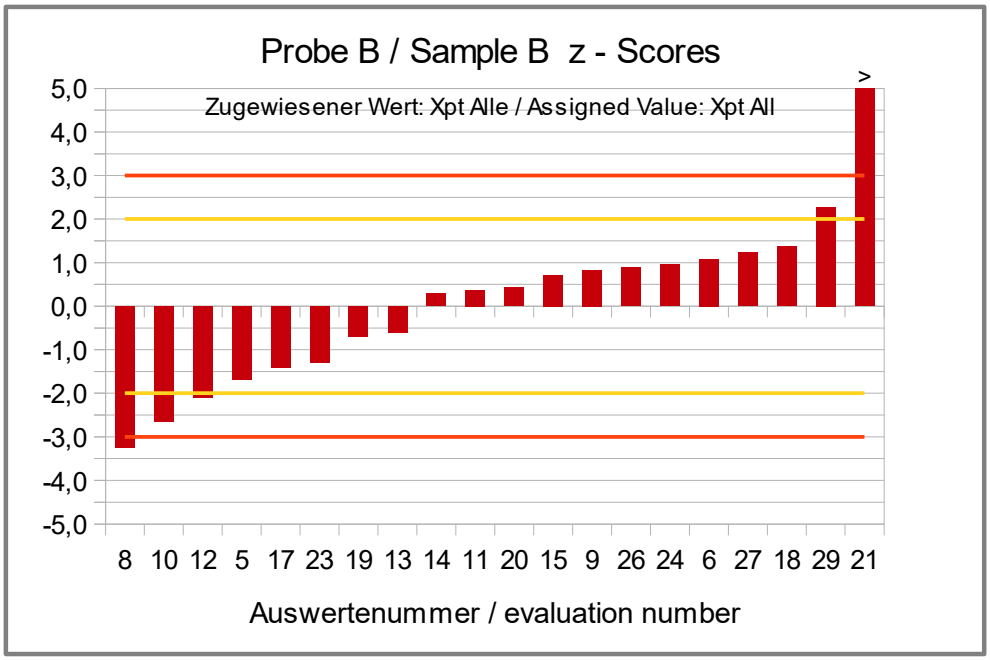


Abb./Fig. 5:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

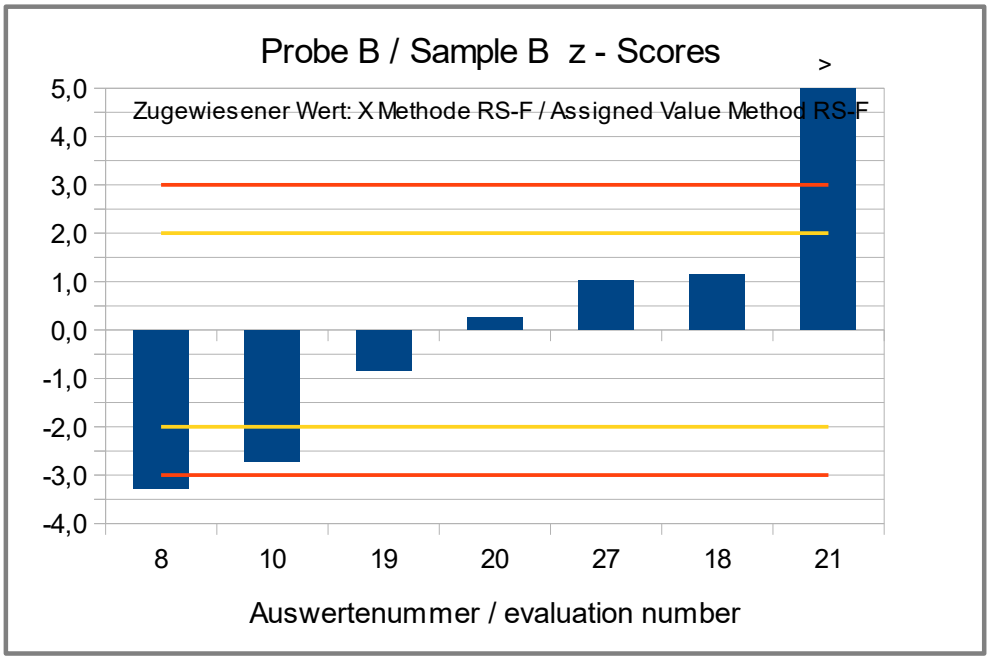


Abb./Fig. 6:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen)

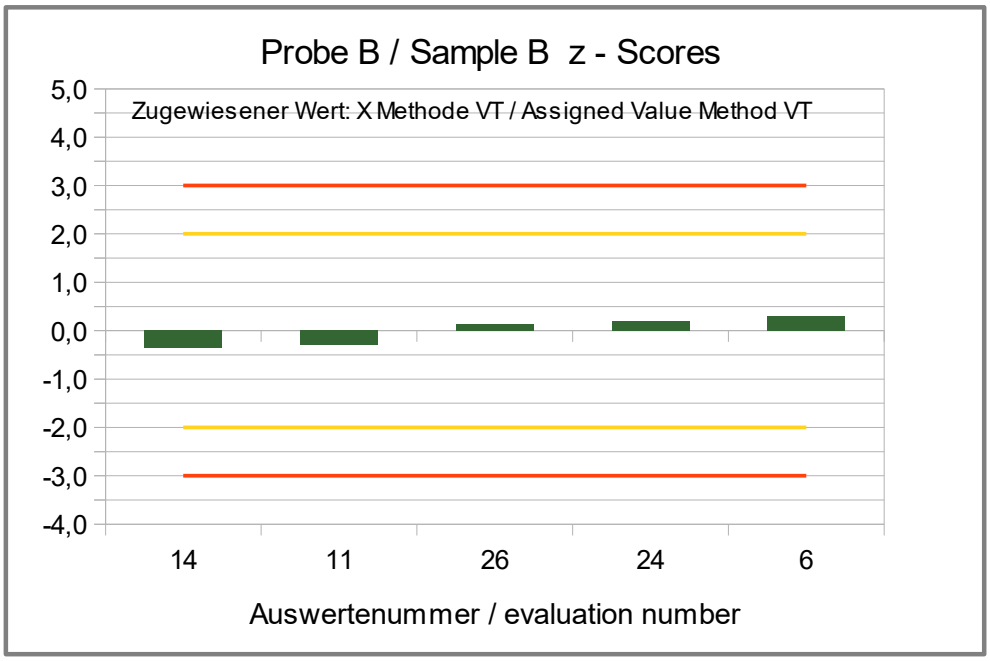


Abb./Fig. 7:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Mustard	Mustard	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	pos/neg	[mg/kg]				
15	positiv	75,5	-0,03		AQ	
13	positiv	71,3	-0,25		BC	
23					BC	
17	positiv	189	5,9		IL	result converted °
12	positiv	> 60			OS	
2	positiv	>13,5			RS-F	
8	positiv	11,3	-3,4	-3,1	RS-F	
10	positiv	19,7	-3,0	-2,4	RS-F	
16	positiv	>13,5			RS-F	
18	positiv	57,0	-1,0	0,66	RS-F	
19	positiv	36,3	-2,1	-1,0	RS-F	
20	positiv	66,6	-0,50	1,5	RS-F	
21	positiv	199	6,5	12	RS-F	result converted °
27	positiv	51,5	-1,3	0,21	RS-F	
5	positiv	123	2,4		SP	
9	positiv	77,0	0,05		SP	
29	positiv	97,0	1,1		SP	
6	positiv	81,1	0,27		VT	
11					VT	
14	positiv	81,0	0,26		VT	
24	positiv	72,9	-0,17		VT	
26	positiv	96,0	1,0		VT	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
IL = Immunolab
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

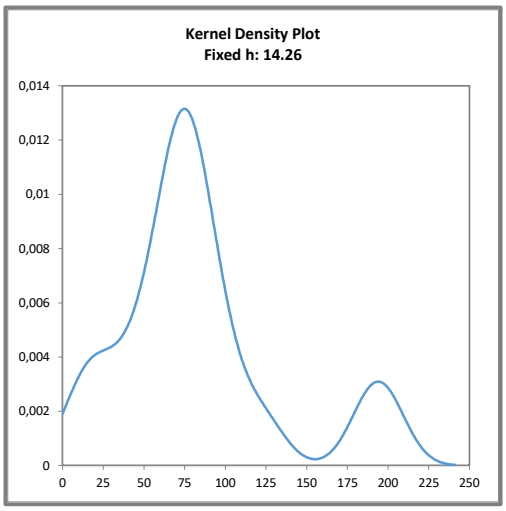


Abb. / Fig. 8:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 a shoulder and a small side peak.

Characteristics: Quantitative evaluation ELISA Mustard

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	17	7
Number of outliers	-	-
Mean	82,6	63,0
Median	75,5	51,5
Robust Mean (X_{pt})	**76,0**	**48,9**
Robust standard deviation (S*)	**39,5**	**34,0**
Target range:		
Target standard deviation σ_{pt}	**19,0**	**12,2**
lower limit of target range	**38,0**	**24,4**
upper limit of target range	**114**	**73,3**
Quotient S/σ_{pt}*	*2,1*	*2,8*
Standard uncertainty $U(X_{pt})$	*12,0*	*16,1*
Results in the target range	*11*	*4*
Percent in the target range	*65*	*57*

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

Comments to the statistical characteristics and assigned values:

The evaluation of all methods as well as of method RS-F showed a slightly increased and increased variability of results, with quotients S^*/σ_{pt} above 2,0 and well above 2,0, respectively. The distribution of results from method RS-F showed a slightly increased variability with a S^*/σ_{pt} >2,0. An evaluation using the z'-Score taking into account the standard uncertainty was dispensed with, since this would make the target area unsuitably large.
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 155% and 100% 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.36).

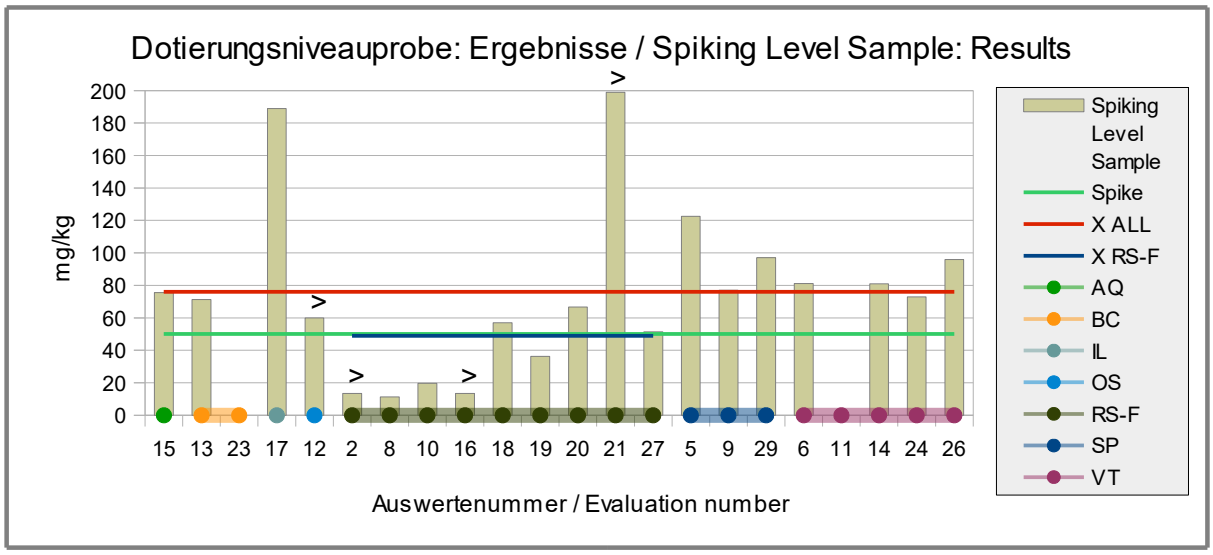


Abb./Fig. 9: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

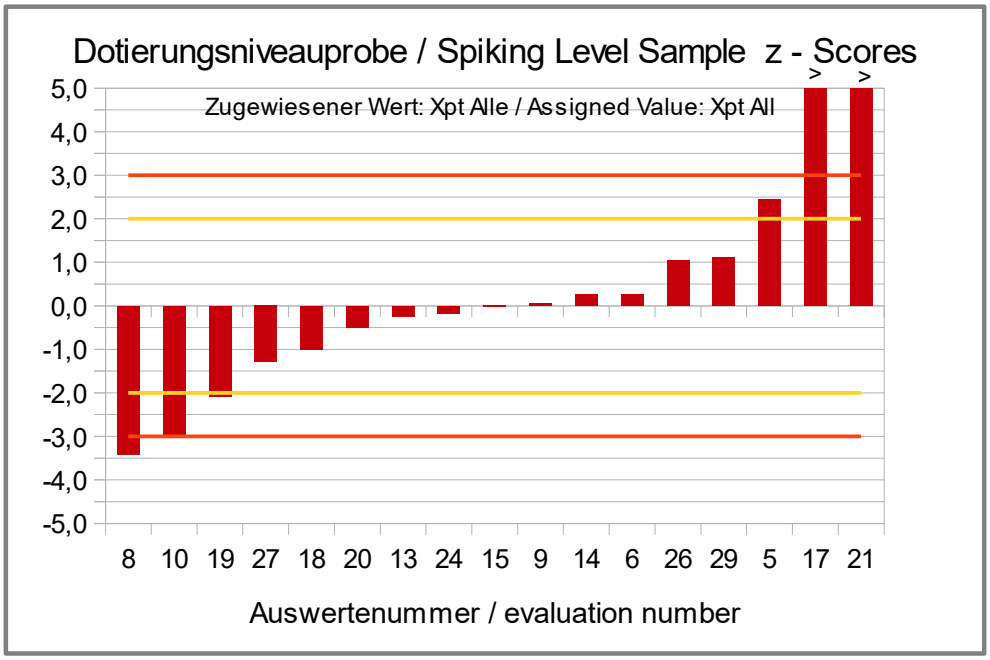


Abb./Fig. 10:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

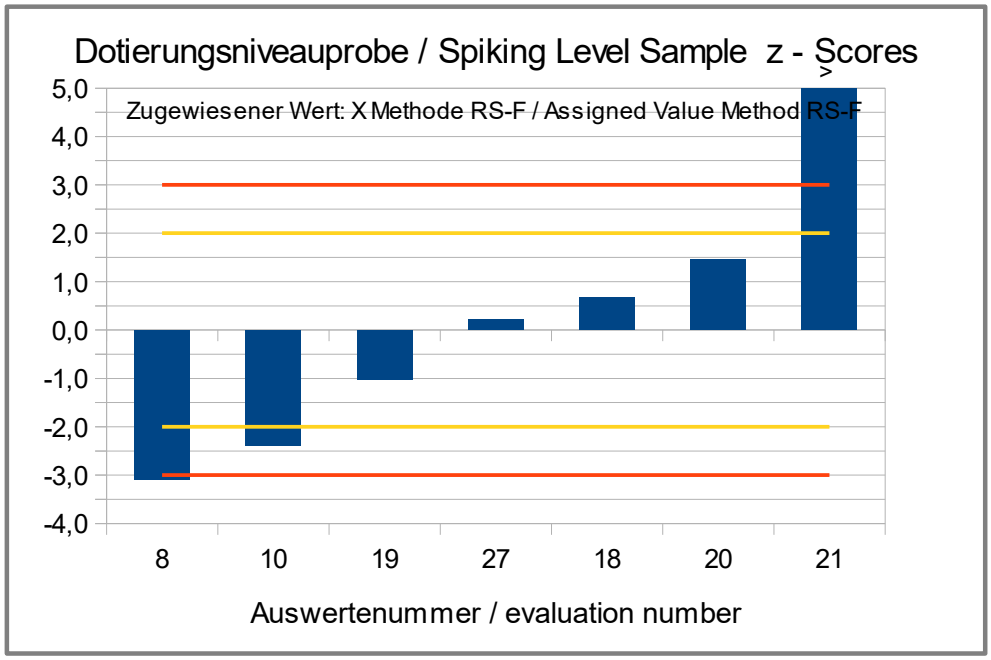


Abb./Fig. 11:
z'-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

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}]		
15	75,5	151	2,0	71,3	**146**	1,8	AQ	
13	71,3	**142**	1,7	51,4	**105**	0,20	BC	
23				40,7	**83**	-0,67	BC	
17	189	377	11	39,1	**80**	-0,80	IL	result converted °
12	> 60			28,7	**59**	-1,7	OS	
2	>13,5			>13,5			RS-F	
8	11,3	22	-3,1	11,4	23	-3,1	RS-F	
10	19,7	39	-2,4	20,4	42	-2,3	RS-F	
16	>13,5			>13,5			RS-F	
18	57,0	**114**	0,55	81,2	166	2,6	RS-F	
19	36,3	**72**	-1,1	49,9	**102**	0,09	RS-F	
20	66,6	**133**	1,3	67,1	**137**	1,5	RS-F	
21	199	397	12	167	342	9,7	RS-F	result converted °
27	51,5	**103**	0,11	79,2	162	2,5	RS-F	
5	123	245	5,8	34,9	**71**	-1,1	SP	
9	77,0	154	2,1	73,0	**149**	2,0	SP	
29	97,0	194	3,7	95,0	194	3,8	SP	
6	81,1	162	2,5	76,9	157	2,3	VT	
11				66,1	**135**	1,4	VT	
14	81,0	162	2,5	65,0	**133**	1,3	VT	
24	72,9	**146**	1,8	75,0	153	2,1	VT	
26	96,0	192	3,7	74,0	151	2,1	VT	

° calculation p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	6	Number in RA	11
Percent in RA	35	Percent in RA	55

* Wiederfindungsrate 100% Bezugsgröße: Senf, s. Seite 5

** Akzeptanzbereich der AOAC für Allergen-ELISAs

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
IL = Immunolab
OS = Orsell
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

For the spiking level sample 35% (6) 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 55% (11) 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		
9	negative		positive		2/2 (100%)	ASU	
20	negative		positive		2/2 (100%)	CEN	
15	negative	-	positive	-	2/2 (100%)	GI	
3	negative		positive		2/2 (100%)	SFA	
4	negative		positive	39,9	2/2 (100%)	SFA	
7	negative		positive		2/2 (100%)	SFA	
8	negative		positive		2/2 (100%)	SFA	
18	negative		positive		2/2 (100%)	SFA	
21	negative	-	positive	-	2/2 (100%)	SFA	
12	negative	< 0,4	positive		2/2 (100%)	SFA-ID	
27	negative	<1	positive	44,6	2/2 (100%)	SFA-ID	
6	negative	N/A	positive	N/A	2/2 (100%)	SFA-Q	
1	negative		positive		2/2 (100%)	div	
22	positive		negative		0/2 (0%)	div	samples interchanged?
25	negative		positive		2/2 (100%)	div	
26a	negative		positive		2/2 (100%)	div	
26b	negative		negative		2/2 (100%)	div	Brassica species negative
26c	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	1		16	
Number negative	17		2	
Percent positive	6		89	
Percent negative	94		11	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, 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 B one negative result was obtained with a method specific for brown and black mustard (result 26b). 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.

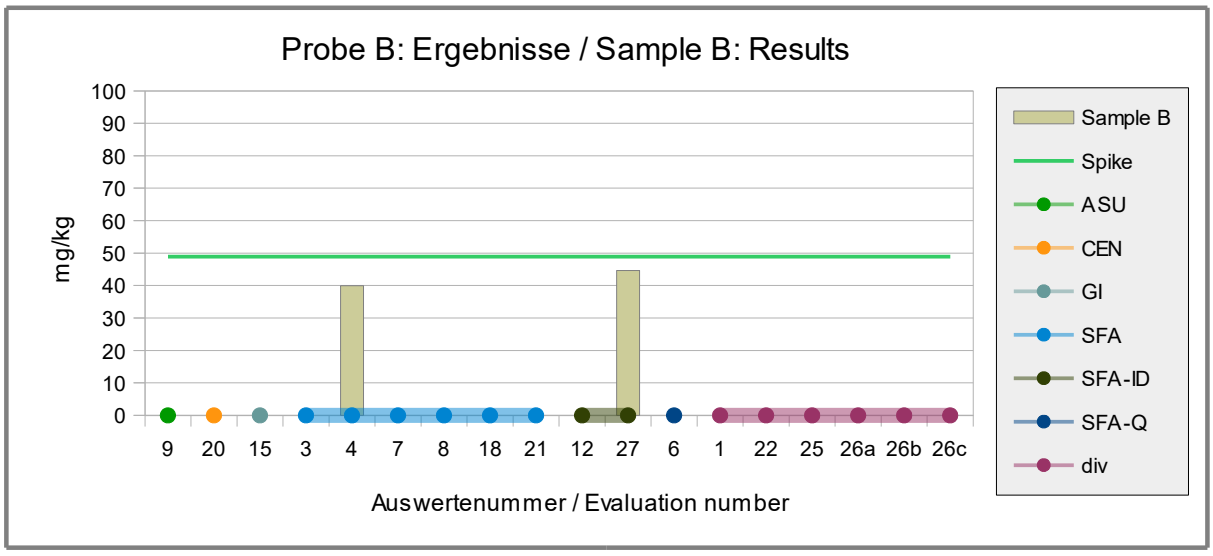


Abb./Fig. 12: PCR Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

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
	[mg/kg]	[mg/kg]			
9	positive			ASU	
20	positive			CEN	
15	positive	-		GI	
3	positive			SFA	
4	positive	12,0		SFA	
7	positive			SFA	
8	positive			SFA	
18	positive			SFA	
21	positive	-		SFA	
12	positive			SFA-ID	
27	positive	26,6		SFA-ID	
6	positive	N/A		SFA-Q	
1	positive			div	
22	positive			div	
25	positive			div	
26a	positive			div	
26b	negative			div	Brassica species negative
26c	positive			div	

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

Methods:

ASU = ASU §64 Methode/method
CEN = European Committee for Standardization Method
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

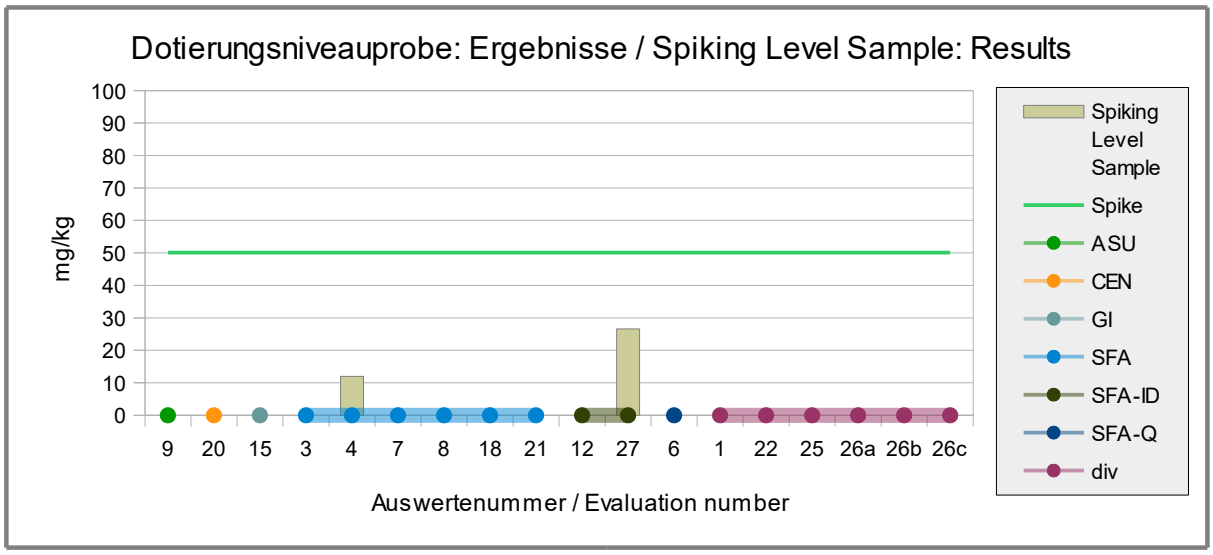


Abb./Fig. 13: PCR-Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

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}]		
9							ASU	
20							CEN	
15	-						GI	
3							SFA	
4	12,0	24	-3,0	39,9	**82**	-0,73	SFA	
7							SFA	
8							SFA	
18							SFA	
21	-						SFA	
12							SFA-ID	
27	26,6	**53**	-1,9	44,6	**91**	-0,35	SFA-ID	
6	N/A			N/A			SFA-Q	
1							div	
22							div	
25							div	
26a							div	
26b							div	Brassica species negative
26c							div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**1**	Number in RA	**2**
Percent in RA	**50**	Percent in RA	**100**

* Wiederfindungsrate 100% Bezugsgröße: Senf, s. Seite 5

** Akzeptanzbereich der AOAC für Allergen-ELISAs

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

CEN = European Committee for Standardization Method

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

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		
15	negative	<2	positive	44,2	2/2 (100%)	AQ	
26a	negative	<2.5	positive	57,0	2/2 (100%)	AQ	
23	negative	<2	positive	42,1	2/2 (100%)	BC	
27	negative	<2	positive	47,1	2/2 (100%)	BC	
13	negative	<2	positive	47,8	2/2 (100%)	BK	
11	negative	<0.51	positive	51,0	2/2 (100%)	ES	result converted °
24	negative	<0.10	positive	84,9	2/2 (100%)	ES	result converted °
10	negative	<LOQ	positive	24,8	2/2 (100%)	IL	
8	negative	< 2	positive	58,9	2/2 (100%)	NL	
2	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
6	negative	<2.5	positive	119	2/2 (100%)	RS-F	
12	negative	< 2,5	positive	>20	2/2 (100%)	RS-F	
14	negative	< 2.5	positive	160	2/2 (100%)	RS-F	
16	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
18	negative	<2.5	positive	161	2/2 (100%)	RS-F	
20	negative		positive	105	2/2 (100%)	RS-F	
26b	negative	<2.5	positive	125	2/2 (100%)	RS-F	
27	negative	<2.5	positive	141	2/2 (100%)	RS-F	
28	negative	<2.5	positive	150	2/2 (100%)	RS-F	
5	negative	<2.0	positive	47,0	2/2 (100%)	SP	
9	negative	<2	positive	54,0	2/2 (100%)	SP	
25	negative		positive	>122	2/2 (100%)	SP	result converted °
29	negative	<0.2	positive	55,0	2/2 (100%)	SP	
26c	negative	<2.5	negative	<2.5	1/2 (50%)	VT	no positive sample detected

° calculation p. 20

	Sample A		Sample B	
Number positive	0		23	
Number negative	24		1	
Percent positive	0		96	
Percent negative	100		4	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BK = BioKits, Neogen
IL = Immunolab
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 B.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Sesame	z-Score Xpt_{50}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
15	44,2	-0,49		AQ	
26a	57,0	0,52		AQ	
23	42,1	-0,66		BC	
27a	47,1	-0,27		BC	
13	47,8	-0,21		BK	
11	51,0	0,05		ES	result converted °
24	84,9	2,7		ES	result converted °
10	24,8	-2,0		IL	
8	58,9	0,67		NL	
2	>20			RS-F	
6	119		-0,54	RS-F	
12	>20			RS-F	
14	160		0,66	RS-F	
16	>20			RS-F	
18	161		0,70	RS-F	
20	105		-0,94	RS-F	
26b	125		-0,36	RS-F	
27b	141		0,10	RS-F	
28	150		0,37	RS-F	
5	47,0	-0,27		SP	
9	54,0	0,28		SP	
25	>122			SP	result converted °
29	55,0	0,36		SP	
26c	<2.5			VT	no positive sample detected

° calculation p. 20

Methoden:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

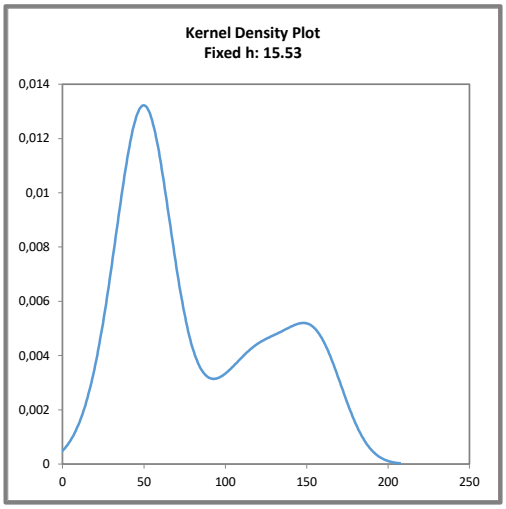


Abb. / Fig. 14:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a nearly symmetrical main peak at approx. 50 mg/kg and a smaller peak at approx. 137 mg/kg due to the results of method RS-F.

Characteristics: Quantitative evaluation ELISA Sesame

Sample B

Statistic Data	**Methods Peak 50** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{50}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	12	7
Number of outliers	-	0
Mean	51,1	137
Median	49,4	141
Robust Mean (X_{pt})	**50,4**	**137**
Robust standard deviation (S*)	**8,41**	**24,5**
Target range:		
Target standard deviation σ_{pt}	**12,6**	**34,3**
lower limit of target range	**25,2**	**68,6**
upper limit of target range	**75,6**	**206**
Quotient S/σ_{pt}*	*0,67*	*0,71*
Standard uncertainty $U_{(Xpt)}$	*3,04*	*11,6*
Results in the target range	*11*	*7*
Percent in the target range	*92*	*100*

Methods:
Peak 50 = AgraQuant, BioCheck, BioKits, ELISA Systems, Immunolab, nutriLinia®, SensiSpec
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 assigned to the respective peak ("peak 50" and "peak 137" = method RS-F) (Assignment see above below the table).

The distributions of the results of peak 50 and of method RS-F showed a low variability of results, with a quotients S/σ_{pt} below 1,0 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 106% and 288% 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.51).

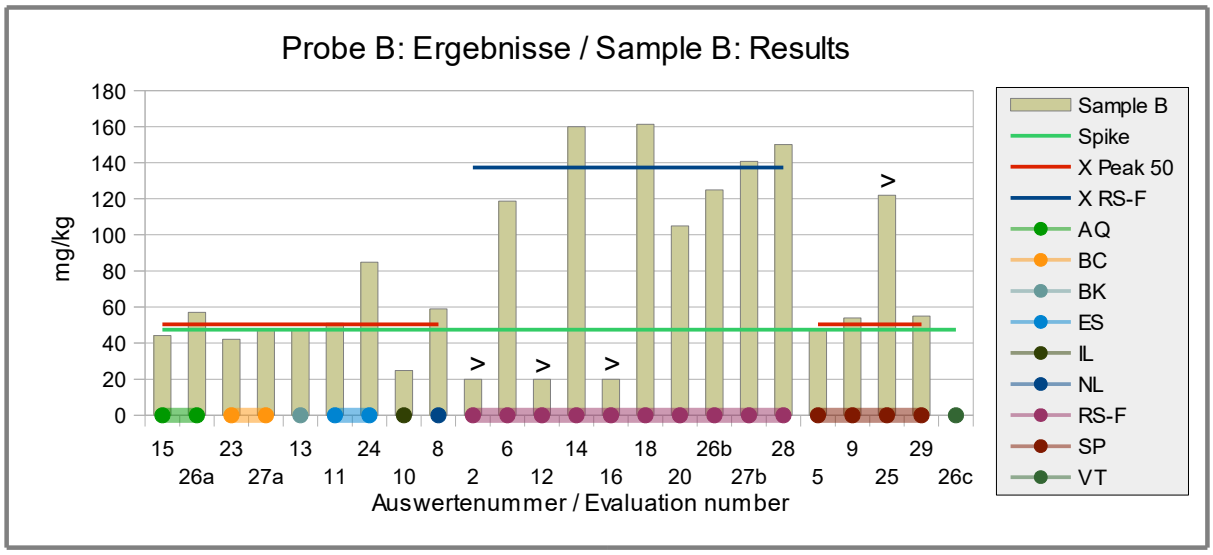


Abb./Fig. 15: ELISA Results Sesame
green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 50"
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

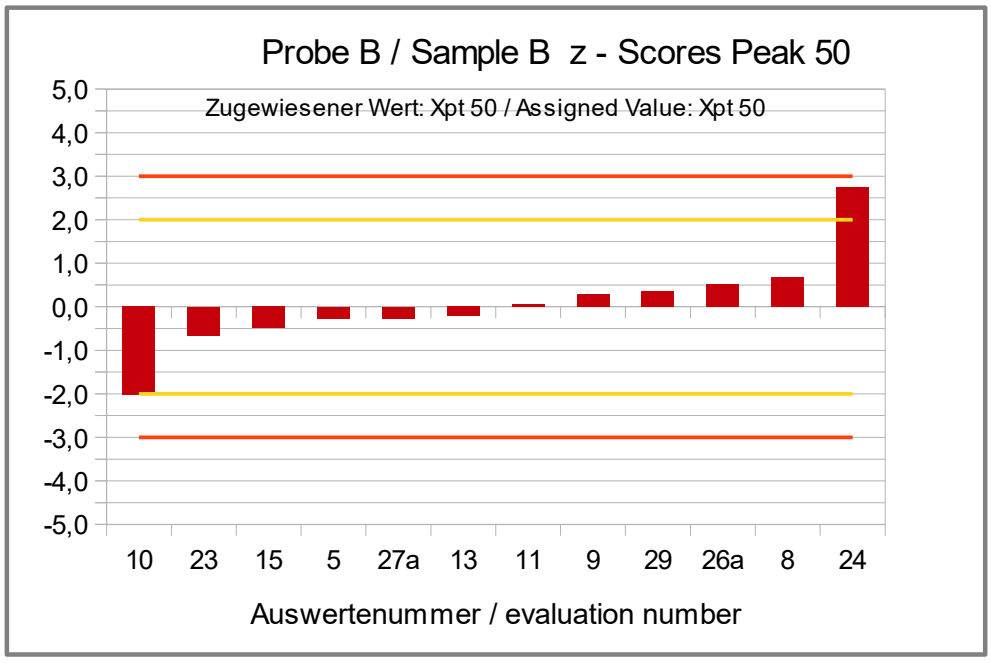


Abb./Fig. 16:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 50

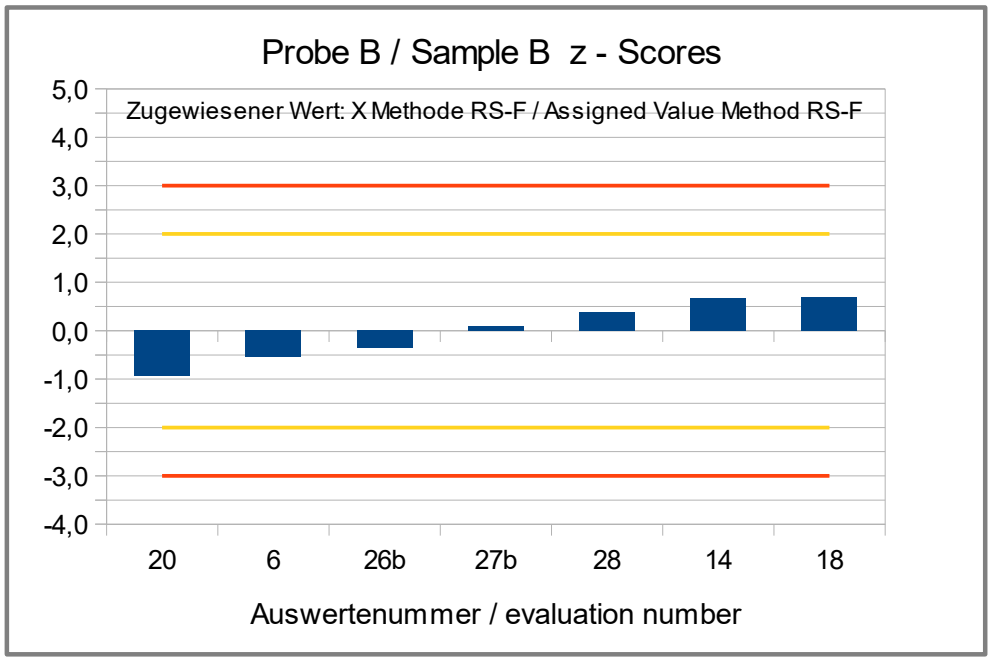


Abb./Fig. 17:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative Valuation of results: Spiking level sample

Evaluation number	Sesame	Sesame	z-Score Xpt_{37}	z-Score $Xpt_{RS\text{-}F}$	z-Score Xpt_{VT}	Method	Remarks
	pos/neg	[mg/kg]					
15	positiv	29,7	-0,80			AQ	
26a	positiv	40,0	0,31			AQ	
23	positiv	33,8	-0,35			BC	
27a	positiv	35,6	-0,16			BC	
13	positiv	32,0	-0,55			BK	
11						ES	result converted °
24	positiv	58,0	2,3			ES	result converted °
10	positiv	45,4	0,89			IL	
8	positiv	39,4	0,25			NL	
2	positiv	>20				RS-F	
6	positiv	99,2		-0,40	-0,38	RS-F	
12	positiv	> 20				RS-F	
14	positiv	210		3,6	3,7	RS-F	
16	positiv	>20				RS-F	
18	positiv	120		0,37	0,39	RS-F	
20	positiv	75,2		-1,3	-1,3	RS-F	
26b	positiv	100		-0,37	-0,35	RS-F	
27b	positiv	115		0,17	0,19	RS-F	
28	positiv	111		0,03	0,05	RS-F	
5	positiv	21,0	-1,7			SP	
9	positiv	38,0	0,10			SP	
25	positiv	>122				SP	result converted °
29	positiv	40,0	0,31			SP	
26c	positiv	116		0,21		VT	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

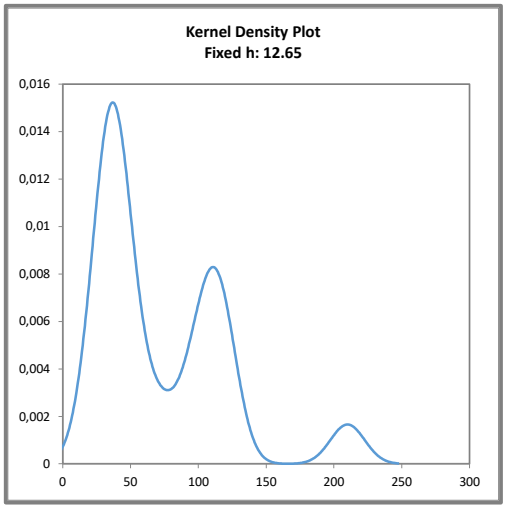


Abb. / Fig. 18:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows two peaks at approx. 37 mg/kg and 110 mg/kg, both with a nearly symmetrical distribution of results.
The higher values ("peak 110") are due to results of the methods RS-F and VT and were therefore evaluated separately.
In addition, there is a peak of a result outside of the target range.

Characteristics: Quantitative evaluation ELISA Sesame

Spiking Level Sample

Statistic Data	**Methods Peak 37** *[mg/kg]*	**Methods Peak 110** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{37}}}$	$\boldsymbol{X_{pt_{110}}}$	$\boldsymbol{X_{pt_{METHOD\ RS\text{-}F}}}$
Number of results	11	8	7
Number of outliers	0	-	-
Mean	37,5	118	119
Robust Mean	38,0	113	111
Median (X_{pt})	**37,1**	**110**	**110**
Robust standard deviation (S*)	**7,51**	**19,3**	**25,0**
Target range:			
Target standard deviation σ_{pt}	**9,27**	**27,6**	**27,4**
lower limit of target range	**18,5**	**55,1**	**54,8**
upper limit of target range	**55,6**	**165**	**164**
Quotient S/σ_{pt}*	*0,81*	*0,70*	*0,91*
Standard uncertainty $U_{(Xpt)}$	*2,83*	*8,54*	*11,8*
Results in the target range	*10*	*7*	*6*
Percent in the target range	*91*	*88*	*86*

Method:
Peak 37 = AgraQuant, BioCheck, BioKits, ELISA Systems, Immunolab, nutriLinia®, SensiSpec
Peak 110 = 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 37" and another assigned to "peak 110" (Assignment see above below the table).
The distributions of the results of peak 37 and peak 110 as well as of method RS-F showed low variabilities of results, with a quotients S/σ_{pt} below 1,0 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 109%, 324% and 324% 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.51).

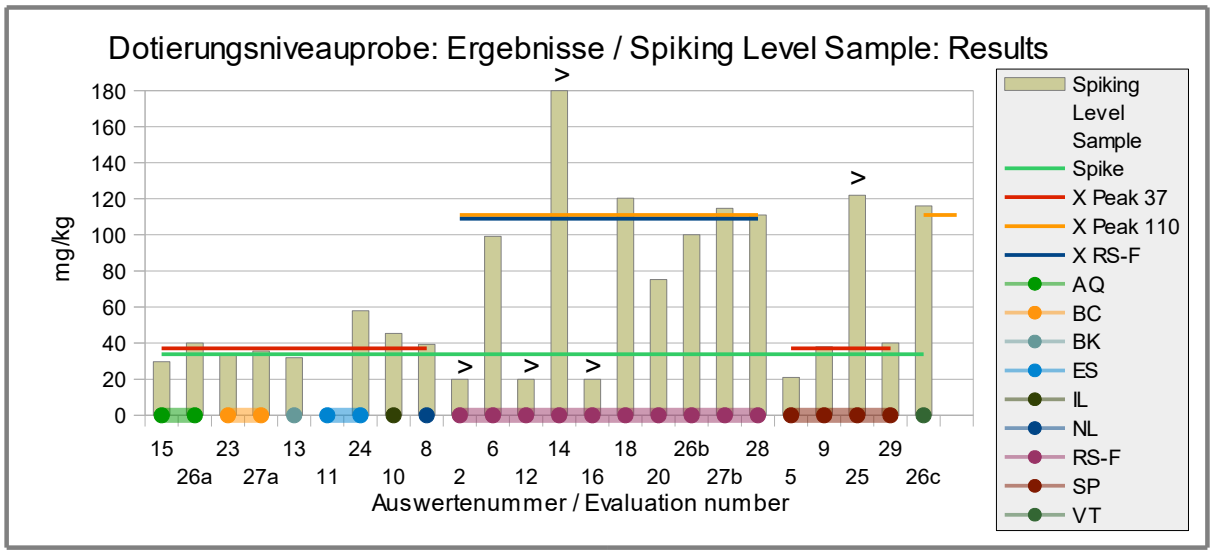


Abb./Fig. 19: ELISA Results Sesame

green line = Spiking level (Spike)
red line = Assigned value robust mean all results of "peak 37"
yellow line = Assigned value robust mean all results of "peak 110"
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

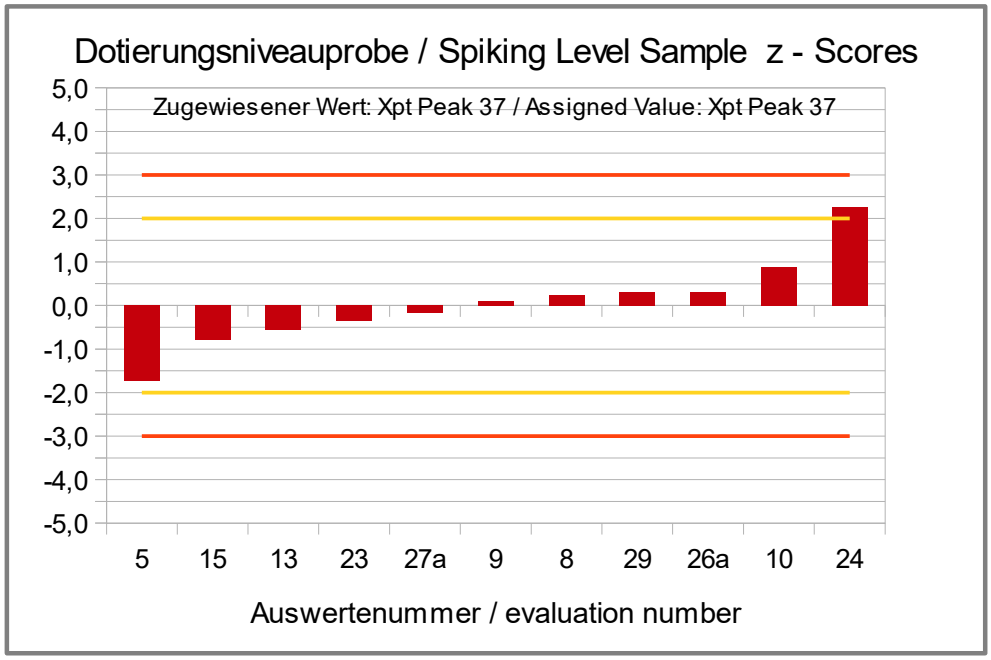


Abb./Fig. 20:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 37

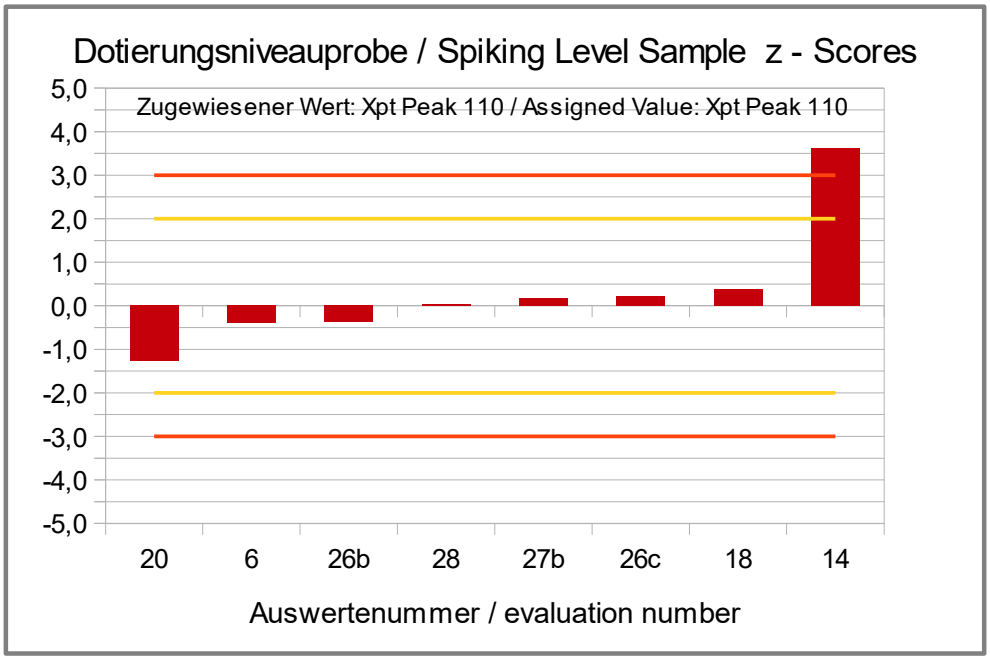


Abb./Fig. 21:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of all results of peak 110

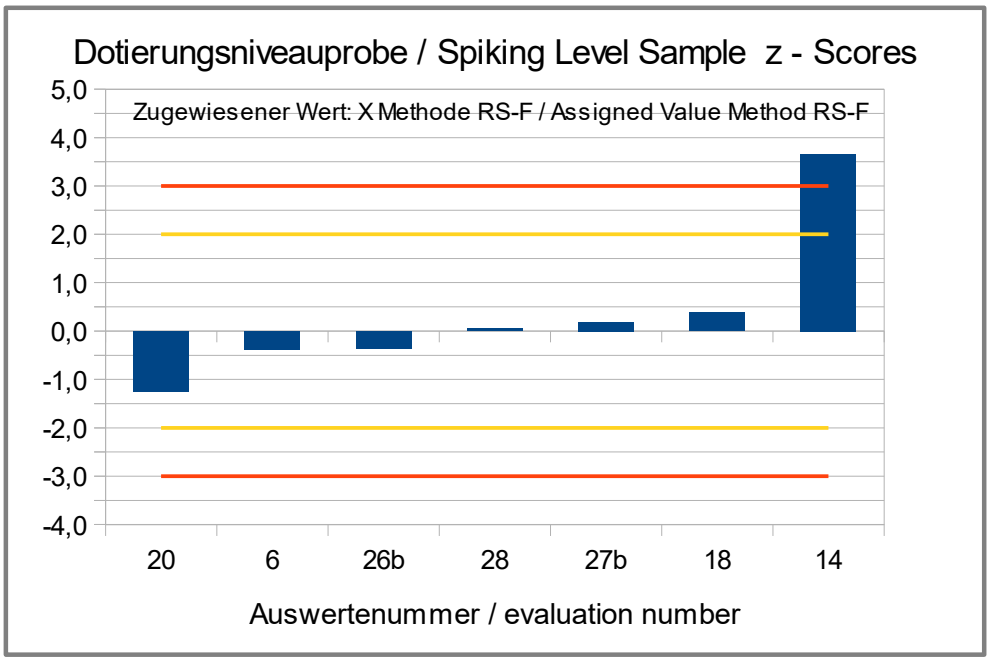


Abb./Fig. 22:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

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}]		
15	29,7	**88**	-0,50	44,2	**93**	-0,28	AQ	
26a	40,0	**118**	0,72	57,0	**120**	0,80	AQ	
23	33,8	**100**	-0,01	42,1	**89**	-0,45	BC	
27a	35,6	**105**	0,20	47,1	**99**	-0,04	BC	
13	32,0	**94**	-0,22	47,8	**101**	0,03	BK	
11				51,0	**107**	0,29	ES	result converted °
24	58,0	171	2,8	84,9	179	3,1	ES	result converted °
10	45,4	**134**	1,4	24,8	**52**	-1,9	IL	
8	39,4	**116**	0,65	58,9	**124**	0,96	NL	
2	>20			>20			RS-F	
6	99,2	293	7,7	119	250	6,0	RS-F	
12	> 20			>20			RS-F	
14	210	619	21	160	337	9,5	RS-F	
16	>20			>20			RS-F	
18	120	355	10	161	340	9,6	RS-F	
20	75,2	222	4,9	105	221	4,8	RS-F	
26b	100	295	7,8	125	263	6,5	RS-F	
27b	115	339	9,5	141	296	7,8	RS-F	
28	111	327	9,1	150	316	8,6	RS-F	
5	21,0	**62**	-1,5	47,0	**99**	-0,04	SP	
9	38,0	**112**	0,48	54,0	**114**	0,55	SP	
25	>122			>122			SP	result converted °
29	40,0	**118**	0,72	55,0	**116**	0,63	SP	
26c	116	342	9,7	<2.5			VT	

° calculation p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**10**	Number in RA	**11**
Percent in RA	**53**	Percent in RA	**58**

* Wiederfindungsrate 100% Bezugsgröße: Sesam, s. Seite 5

** Akzeptanzbereich der AOAC für Allergen-ELISAs

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BK = BioKits, Neogen
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

53% (10) 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 58% (11) of the recovery rates were within the range of acceptance. All of the results which were in the range of acceptance were obtained by methods AQ, BC, BK, ES, IL, NL and SP.

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		
9	negative		positive		2/2 (100%)	ASU	
15	negative	-	positive	-	2/2 (100%)	GI	
4	negative		positive	23,7	2/2 (100%)	SFA	
7	negative		positive		2/2 (100%)	SFA	
8	negative		positive		2/2 (100%)	SFA	
21	negative	-	positive	-	2/2 (100%)	SFA	
12	negative	< 0,4	positive		2/2 (100%)	SFA-ID	
27	negative	<1	positive	20,4	2/2 (100%)	SFA-ID	
1	negative		positive		2/2 (100%)	div	
20	negative		positive		2/2 (100%)	div	
22	positive		negative		0/2 (0%)	div	samples interchanged?
26	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	1		11	
Number negative	11		1	
Percent positive	8		92	
Percent negative	92		8	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
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.

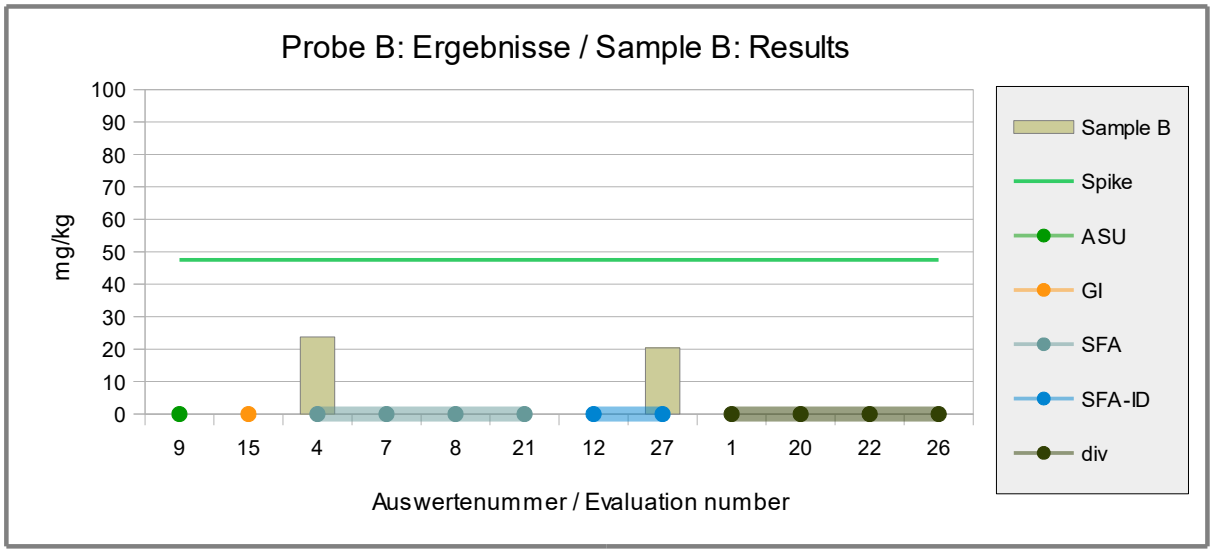


Abb./Fig. 23: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

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
	[mg/kg]	[mg/kg]			
9	positiv			ASU	
15	positiv	-		GI	
4	positiv	5,64		SFA	
7	positiv			SFA	
8	positiv			SFA	
21	positiv	-		SFA	
12	positiv			SFA-ID	
27	positiv	22,6		SFA-ID	
1	positiv			div	
20	positiv			div	
22	positiv			div	
26	positiv			div	

Number positive	12	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positiv	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

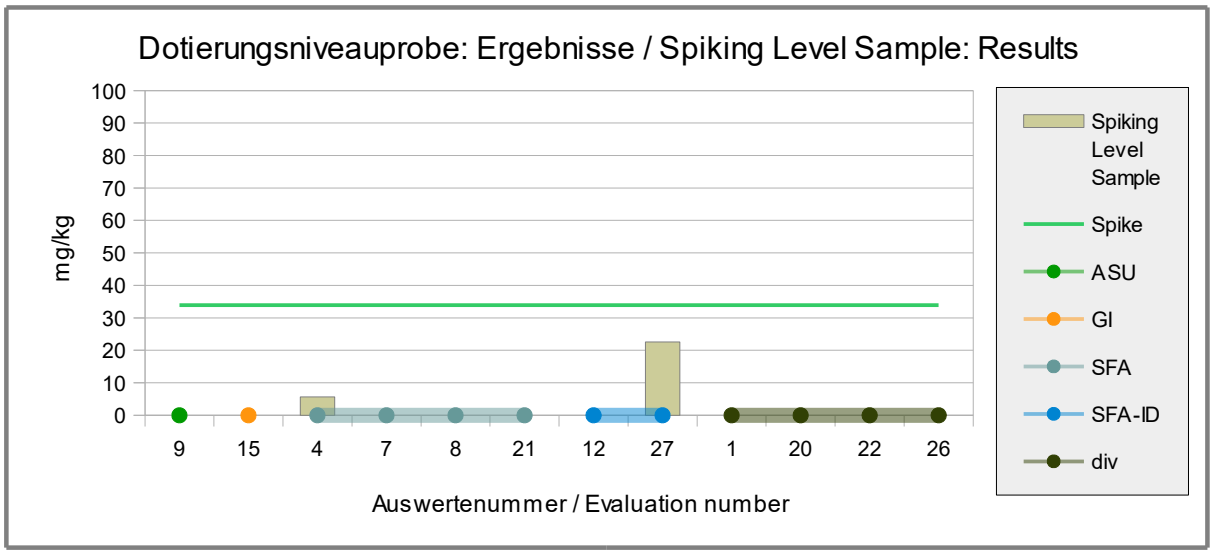


Abb./Fig. 24: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

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}]		
9							ASU	
15	-			-			GI	
4	5,64	17	-3,3	23,7	**50**	-2,0	SFA	
7							SFA	
8							SFA	
21	-			-			SFA	
12							SFA-ID	
27	22,6	**67**	-1,3	20,4	43	-2,3	SFA-ID	
1							div	
20							div	
22							div	
26							div	

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

* Wiederfindungsrate 100% Bezugsgröße: Sesam, s. Seite 5

** Akzeptanzbereich der AOAC für Allergen-ELISAs

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

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:

In each case one of the two participants received a recovery rate in the range of the AOAC-recommendation of 50-150% with the spiking level sample or with the spiked food matrix sample B by PCR.

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

4.4 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 50“ or „Peak 37“)		ELISA Sesame: Xpt („Peak 110“)		ELISA Sesame: Xpt (Methode: RS-F)	
	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1												
2												
3												
4												
5	-1,7	2,4					-0,27	-1,7				
6	1,1	0,27			0,31					-0,40	-0,54	-0,38
7												
8	-3,2	-3,4	-3,3	-3,1			0,67	0,25				
9	0,83	0,1					0,28	0,10				
10	-2,7	-3,0	-2,7	-2,4			-2,0	0,89				
11	0,37				-0,30		0,05					
12	-2,1											
13	0,60	-0,25					-0,21	-0,55				
14	0,30	0,26			-0,36					3,6	0,66	3,7
15	0,71	-0,03					-0,49	-0,80				
16												
17	-1,4	5,9										
18	1,4	-1,0	1,1	0,66						0,37	0,70	0,39
19	-0,70	-2,1	-0,84	1,0								
20	0,44	-0,50	0,25	1,5						-1,3	-0,94	-1,3
21	7,0	6,5	6,6	12								
22												
23	-1,3						-0,66	-0,35				
24	0,96	-0,17			0,20		2,7	2,3				
25												
26/26a	0,89	1,1			0,15		0,52	0,31				
26b										-0,37	-0,36	-0,35
26c										0,21		
27	1,2	-1,3	1,0	0,21			-0,27	-0,16		0,17	0,10	0,19
28										0,03	0,37	0,05
29	2,3	1,1					0,36	0,31				

Methods: RS-F = Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
Peak 50 / Peak 37 = AgraQuant, BioCheck, BioKits, ELISA Systems, Immunolab, nutriLinia®, SensiSpec
Peak 110 = Ridascreen® Fast, Veratox

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	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample	Sample B	Spiking Level Sample
1										
2										
3										
4							-0,73	-3,0	-2,0	-3,3
5	5,8	-1,1	-0,04	-1,5						
6	2,3	2,5	6,0	7,7						
7										
8	-3,1	-3,1	0,96	0,65						
9	2,0	2,1	0,55	0,48						
10	-2,3	-2,4	-1,9	1,4	-4,0	-3,9				
11	1,4		0,29							
12	-1,7									
13	0,20	1,7	0,03	-0,22						
14	1,3	2,5	9,5	21						
15	1,8	2,0	-0,28	-0,50						
16										
17	-0,80	11			-4,0	-3,7				
18	2,6	0,55	9,6	10						
19	0,09	-1,1								
20	1,5	1,3	4,8	4,9						
21	9,7	12								
22										
23	-0,67		-0,45	-0,01						
24	2,1	1,8	3,1	2,8						
25										
26/26a	2,1	3,7	0,80	0,72						
26b			6,5	7,8						
26c				9,7						
27/27a	2,5	0,11	-0,04	0,20	-1,9	-1,8	-0,35	-1,9	-2,3	-1,3
27b			7,8	9,5						
28			8,6	9,1						
29	3,8	3,7	0,63	0,72						

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	15	12.07.	negative	<2 ppm	positive	71,3	positive	75,5	1	2	15	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
BC	13	14.07.21	negative	<2	positive	51,4	positive	71,3	2	2	50	Mustard	BioCheck ELISA Mustard-Check
BC	23		-	<2	-	40,73	-	X	1	2	30	Mustard	BioCheck ELISA Mustard-Check
IL	17	21.07.21	-	< 2P	-	12P	-	58P	1	2	28,6	Mustardprotein	Immunolab Mustard ELISA
OS	12		negative	<2	positive	28,7	positive	> 60		2		Mustard	ORSELL MUSTARD KIT
RS-F	2	08.06.21	negative	<0,5	positive	>13,5	positive	>13,5		0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	8	14/06	-	< 0,5	-	11,35	-	11,25		0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	10	30.06.21	negative	<LOQ	positive	20,36	positive	19,68	0,1	0,5		mustard flour	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	16	14.06.21	-	<0,5	-	>13,5	-	>13,5		0,5		mustard	ridascreen fast mustard R6152
RS-F	18	02.07.21		<0.5	-	81,19	-	56,99		0,5	30,94	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	19	04.06.21	-	<0.50	-	49,94	-	36,31	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	20		negative		positive	67,11	positive	66,59	0,5	1,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	21	15.07.21	negative	< 0,5P	positive	51,4P	positive	61,1P	0,1	0,5	40	Mustardprotein	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	27	16.06.21	negative	<0.5	positive	79,16	positive	51,48	0,5	0,5	18,81	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
SP	5	09.06.21	negative	<2.0	positive	34,9	positive	122,6		2	50	Mustard	SensiSpec ELISA Mustard, Eurofins
SP	9	23.06.21	negative	<2	positive	73	positive	77	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins
SP	29	01.06.21	negative	<1	positive	95	positive	97	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins
VT	6	08.06.21	negative	<2.5	positive	76,94	positive	81,11	N/A	2,5	N/A	Mustard	Veratox Mustard, Neogen
VT	11	17.06.21	negative	<1.0	positive	66,1	Not tested		1	2,5		Mustard	Veratox Mustard, Neogen
VT	14	16/6/21	-	< 2.5	-	65	-	81	< 1	2,5	31	Mustard	Veratox Mustard, Neogen
VT	24	16/07	ND	<2.5	D	75	D	72,9		2,5		mustard	Neogen Veratox
VT	26	17.06.2021, 29.06.2021	negative	<2.5	positive	74	positive	96	2,5	2,5		Mustard	Veratox Mustard, Neogen

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

Continuation ELISA Mustard:

Meth. 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	15			yes	
BC	13		0.5g Sample/10ml kit extraction buffer/ 15mins at 60C	yes	
BC	23			yes	
IL	17			yes	
OS	12			yes	
RS-F	2				
RS-F	8	Specific Ab detect yellow , brow n, black mustard.	as per kit Instructions	yes	
RS-F	10		Result w as out of measuring range and thus extrapolated from standard curve	No	
RS-F	16		MUSTARD EXTRACTION BUFFER, 10 MIN A 60°C	yes	
RS-F	18			yes	Kit has high cross-reactivity to Rapeseed oil. Samples A and B contain 50% rapeseed.
RS-F	19			No	
RS-F	20			yes	LFOD-TSTSOP-8828
RS-F	21	The antibodies used in the test specifically detect different kinds of mustard (yellow , w hite, brow n, black mustard). The results are for mustard, in general.	Preparation of the sample and test implementation follow ing the instruction of RIDASCREEN® FAST Senf/Mustard (Art. Nr.: R6152), Lot 25460 - extraction w ith diluted Allergen Extraction buffer 10 min at 60°C	yes	
RS-F	27	As Per Kit Instructions	As Per Kit Instructions	yes	
SP	5			yes	
SP	9	detects mustard proteins	as per kit instructions	yes	
SP	29				
VT	6		as per kit insert	yes	recovery in sample A 108%
VT	11		Extraction: 60C pre-heated TRIS extraction buffer/ samples extracted in shaking w aterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	yes	
VT	14	Mustard	TRIS-EDTA/15 Minutes/60 C	yes	
VT	24				
VT	26				

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	15	29.06.	negative	<2 ppm	positive	44,2	positive	29,7	0,2	2	15	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
AQ	26a	28.06.21	negative	<2.5	positive	57	positive	40	2,5	2,5		Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
BC	23		-	<2	-	42,11	-	33,8	0,2	2	30	Sesame	BioCheck ELISA Mustard-Check
BC	27	02.06.21	negative	<2	positive	47,05	positive	35,6	2	2	26,64	Sesame	BioCheck ELISA Sesame-Check
BK	13	14.07.21	negative	<2	positive	47,8	positive	32	2	2	50	Sesame	BioKits Sesame Protein Assay Kit, Neogen
ES	11	09.06.21	negative	<0.125 P	positive	12,5P	Not tested		0,125	0,25		Sesameprotein	ELISA Systems Sesame ESSESRD-48
ES	24	16/07	ND	<0.25P	D	20,8P	D	14,2P		0,25		Sesame seed protein	Elisa Systems
IL	10	01.07.21	negative	<LOQ	positive	24,75	positive	45,36		2		Please select!	Immunolab Sesame ELISA
NL	8	15/06	-	< 2	-	58,90	-	39,38		2		Sesame	Sesame-E nutriLinia über RomerLabs
RS-F	2	04.06.21	negative	<2,5	positive	>20	positive	>20		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	6	09.06.21	negative	<2.5	positive	118,78	positive	99,2	N/A	2,5	N/A	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	12		negative	< 2,5	positive	>20	positive	> 20		2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	14	13/7/21	-	< 2.5	-	160	-	210	0,2	2,5	16	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	16	03.06.21	-	<2,5	-	>20	-	>20		2,5		Sesame	ridascreen fast Sesame R7202
RS-F	18	20.07.21	-	<2.5	-	161,3	-	120,3		2,5	27,13	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	20		negative		positive	104,98	positive	75,2	1,2	4		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	26b	22.06.21	negative	<2.5	positive	125	positive	100	2,5	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	27	02.06.21	negative	<2.5	positive	140,7	positive	114,76	2,5	2,5	29,78	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	28	14/07/21	-	<2.5	-	150	-	111	0,2	2,5	29	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
SP	5	09.06.21	negative	<2.0	positive	47	positive	21		2	50	Sesame	SensiSpec ELISA Sesame, Eurofins
SP	9	23.06.21	negative	<2	positive	54	positive	38	1,5	2		Sesame	SensiSpec ELISA Sesame, Eurofins
SP	25	20.07.21	negative		positive	>30P	positive	>30P		2		Sesameprotein	SensiSpec ELISA Sesame, Eurofins
SP	29	01.06.21	negative	<0.2	positive	55	positive	40	0,2	2		Sesame	SensiSpec ELISA Sesame, Eurofins
VT	26c	13.06.21	negative	<2.5	negative	<2.5	positive	116	2,5	2,5		Sesame	Veratox Sesame Allergen, Neogen

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Sesame:

Meth. 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	26a				
BC	23			yes	
BC	27a	As Per Kit Instructions	As Per Kit Instructions	yes	
BK	13		0.5g Sample/10ml kit extraction buffer/ 15mins at 60C	yes	
ES	11	Anti-sesame seed 2S-albumin	and samples extracted in shaking w aterbath @ 60C for 15 min.	yes	
ES	24				
IL	10		Sample spiking level w as further dilluted 1:4	No	
NL	8	Antibodies against sesame proteins	As Per Kit Instructions	yes	
RS-F	2				
RS-F	6		as per kit insert	yes	recovery in sample A 102%
RS-F	12			yes	
RS-F	14	Sesame	Allergen Kit Extraction Buffer/10 minutes/60 C	yes	
RS-F	16		SESAME EXTRACTION BUFFER, 10 MIN A 60°C	NO	
RS-F	18			yes	
RS-F	20			yes	LFOD-TST-SOP-8867
RS-F	26b				
RS-F	27b	As Per Kit Instructions	As Per Kit Instructions	yes	
RS-F	28	sesame protein	AEP, 60C, 10mins, centrifuge 2500g	yes	
SP	5			yes	
SP	9	detects sesame proteins	As Per Kit Instructions	yes	
SP	25		Eurofins test instruction version February 21th, 2019	no	
SP	29				
VT	26c				

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	8	07.06.21	negative		positive		positive					Celery-DNA	ASU §64 Methode/method
ASU	9	17.06.21	negative		positive		positive		10			Celery-DNA	ASU §64 Methode/method
CEN	1		negative		positive		positive		5	nd			CEN/TS 15634-2
CEN	20		negative		positive		positive		10			Please select!	Selection PCR-Methods
FP	10	28.06.21	negative	negative at the LOD	positive	0,16	positive	0,82	0,1	0,8		Celery-DNA	foodproof Detection Kit, BIOTECON Diagnostics
FP	15	19.06.	negative	<0,1 ppm	positive	<0,8 ppm	positive	<0,8 ppm	0,1	0,8	30	Celery	foodproof Detection Kit, BIOTECON Diagnostics
FP	17	19.07.21	negative	< 0,080	positive	0,58	positive	3,1	0,08	0,8	16,1	Celery-DNA	foodproof Detection Kit, BIOTECON Diagnostics
IM	3	04.06.21	negative		positive		positive		0,4			Please select!	Other: IMEGEN
SFA	7	14.06.21	negative		positive		positive		0,4	1		Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16	11.06.21	NEGATIVO		POSITIVO		POSITIVO		2			CELERY DNA	SURE FOOD ALLERGEN, R-BIOPHARM CONGEN CELERY
SFA	21	06.07.21	negative	-	positive	-	positive	-	0,4	-	-	Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18a	21. Jul	negative		positive		positive		0,4			Celery-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	4		negative		positive		positive					Celery-DNA	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	12		negative	< 0,4	positive		positive		0,4			Celery-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	27	08.06.21	negative	<1	positive	25,43	positive	20,52	1	1	44,65	Celery	Sure Food Allergen ID, R-Biopharm / Congen
div	18b	21.07.21	negative		negative		negative		1			Celery-DNA	Selection PCR-Methods
div	22		positive		negative		positive		0,008	0,08		Please select!	internal Methods
div	25		Negative		Positive		Positive		see note			Please select!	Real Time PCR Internal Method: MEB65
div	26	20.08.20	negative		positive	3	positive	2			40	Celery seed, dried	in house

* NWG Nachweisgrenze / 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	8	Protein of mannitol dehydrogenase	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
ASU	9		CTAB / Proteinase K /Amylase A / Promega Maxwell / realtime PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-56:2014-08
CEN	1	Manitol déshydrogenase	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
CEN	20		EN 15634-2:2019 - Real time PCR	no	LFOD-TST-SOP-8859
FP	10		Absolute quantification was performed using standard curve 28062021, with Allergen RM 800 reference material.	No	LOD was determined to be 1 celery genome equivalent and 0.1 ppm in a celery-spiked rice flour matrix. The LOQ was determined to be 0.8 ppm based on the threshold set by the standard curve.
FP	15			yes	
FP	17		real time PCR, foodproof DNA Extraktion Biotecon Diagnostics	yes	
IM	3			yes	
SFA	7	Sellerie	CTAB Präzipitation, QIAgen PCR Purification Kit, Real Time PCR	nein	
SFA	16		PREP ADVANCE SUREFOOD/TQ POLYMERASE/RT PCR/45 CYCLES	yes	
SFA	21		The test used is a real-time PCR for the direct, qualitative detection of specific celery (Apium graveolens) DNA sequences. DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 22490	yes	SureFood® ALLERGEN Celery - Art. S3605
SFA	18a	Celery		No	
SFA-4p	4		SureFood®PREP Advanced Kit, Protokoll 1	yes	
SFA-ID	12			yes	
SFA-ID	27	As Per Kit Instructions	As Per Kit Instructions	yes	
div	18b	Celery	In House Method	yes	
div	22	ribosomal RNA		yes	
div	25	Mannitol dehydrogenase (MDH)	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (50 cycles of amplification)	yes	LD PCR=15 pg DNA (<10mg/kg for reference material)
div	26	M6PR-Gene			

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	9	17.06.21	negative		positive		positive		5			Mustard-DNA	ASU §64 Methode/method
CEN	20		negative		positive		positive		10			Please select!	Selection PCR-Methods
GI	15	12.07.	negative	-	positive	-	positive	-				Mustard-DNA	GEN-IAL First Allergen
SFA	3	04.06.21	negative		positive		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	4		negative		positive	39,94	positive	11,99	0,4	1		Mustard	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	10.06.21	negative		positive		positive		0,4	1		Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	08.06.21	negative		positive		positive					Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18	06.07.21	negative		positive		positive		0,4			Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	06.07.21	negative	-	positive	-	positive	-	0,4	-	-	Mustard-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	12		negative	< 0,4	positive		positive		0,4			Mustard-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	27	08.06.21	negative	<1	positive	44,62	positive	26,62	1	1	34,67	Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	6	08.06.21	negative	N/A	positive	N/A	positive	N/A	N/A		N/A	Mustard-DNA	Sure Food Allergen Quant, R-Biopharm / Congen
div	1		negative		positive		positive		5	nd			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	22		positive		negative		positive		0,008	0,08		Please select!	internal Methods
div	25		Negative		Positive		Positive		see note			Please select!	Real Time PCR Internal Method: MEB67
div	26a	08.07.21	negative		positive		positive					Mustard-DNA (Sinapis alba)	
div	26b	08.07.21	negative		negative		negative					Mustard-DNA (Brassica nigra, Brassica juncea)	
div	26c	08.07.21	negative		positive		positive					Mustard-DNA	

* 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	9		CTAB / Proteinase K /Amylase A / Promega Maxwell / realtime PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-65:2017-10
CEN	20		CEN/TS 15634-5:2016 - Real time PCR	yes	LFOD-TST-SOP-8858
GI	15			yes	
SFA	3			yes	
SFA	4		SureFood®PREP Advanced Kit, Protokoll 1	yes	
SFA	7	Mustard	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
SFA	8	characteristic sequence part of Mustard-DNA	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
SFA	18	Mustard		No	
SFA	21		The test detects DNA of white mustard (Sinapis alba), indian mustard (Brassica juncea) und black mustard (Brassica nigra). The results are for mustard, in general. DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 22490	yes	SureFood® ALLERGEN Mustard - Art. S3609
SFA-ID	12			yes	
SFA-ID	27	As Per Kit Instructions	As Per Kit Instructions	yes	
SFA-Q	6		cleaning using SureFood Prep Advanced S1053, real time PCR, 45 cycles	yes	Kit # S3609
div	1	MADS-D	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	22	MADS D		yes	
div	25	MADS D protein gene,Reverse transcriptase from gypsy-like retroelement 13G42-26	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (50 cycles of amplification)	yes	LD PCR=15 pg DNA (<10mg/kg for reference material)
div	26a	cDNA Sequence MADS-D Protein			
div	26b	RT Gen Gypsylike Retro13G42-26			
div	26c	Cruciferin A Gene			

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	9	17.06.21	negative		positive		positive		10			Sesame-DANN	ASU §64 Methode/method
GI	15	29.06.	negative	-	positive	-	positive	-				Sesame-DNA	GEN-IAL First Allergen
SFA	4		negative		positive	23,72	positive	5,64	0,4	1		Sesame	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	10.06.21	negative		positive		positive		0,4	1		Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	08.06.21	negative		positive		positive					Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	06.07.21	negative	-	positive	-	positive	-	0,4	-	-	Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	12		negative	< 0,4	positive		positive		0,4			Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	27	08.06.21	negative	<1	positive	20,38	positive	22,57	1	1	40	Please select!	Sure Food Allergen ID, R-Biopharm / Congen
div	1		negative		positive		positive		5	nd			Waiblinger H-U - Ring trial validation of single and multiplex real-time PCR methods for the detection and quantification of the allerginic food ingredients Sesame, almond, lupine and Brazil nur - J. Verbr. Lebensm. - DOI 10,1007/s00003-014-0868-x
div	20		negative		positive		positive		10			Please select!	Selection PCR-Methods
div	22		positive		negative		positive		0,008	0,08		Please select!	internal Methods
div	26	08.07.21	negative		positive		positive					Sesame-DNA	

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation PCR Sesame:

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	9		CTAB / Proteinase K /Amylase A / Promega Maxwell / realtime PCR / 45 Cycles	yes	§ 64 LFGB L 18.00-19:2014-08
GI	15	2S Albumin gene		yes	
SFA	4		SureFood®PREP Advanced Kit, Protocol 1	yes	
SFA	7	Sesame	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes	
SFA	8	charakteristischer Sequenzabschnitt der Sesam-DNA	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
SFA	21		The test used is a real-time PCR for the direct, qualitative detection of specific sesame (Sesamum indicum) DNA sequences. DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 22490	yes	SureFood® ALLERGEN Sesame - Art. S3608
SFA-ID	12			yes	
SFA-ID	27	As Per Kit Instructions	As Per Kit Instructions	yes	Reported as Sesame
div	1	Albumine 2S	Extraction kit: NucleoSpin Food Macherez-Nagel - Real-time PCR 40 cycles	yes	
div	20		Realtime PCR in house method	no	LFOD-TST-SOP-8733
div	22	2S albumine		yes	
div	26	Internal 2SAlbumine Gene			

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA-ptAL04 Spiking Level Sample

Weight whole sample	1,07	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	45	18,0
2	4,97	46	18,5
3	5,02	46	18,3
4	5,01	58	23,2
5	4,98	46	18,5
6	4,98	54	21,7
7	4,98	57	22,9
8	4,99	51	20,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	50,4	Particles
Standard deviation	5,38	Particles
χ^2 (CHI-Quadrat)	4,03	
Probability	**78**	%
Recovery rate	96	%

Normal distribution		
Number of samples	8	
Mean	20,2	mg/kg
Standard deviation	2,16	mg/kg
rel. Standard deviaton	10,7	%
Horwitz standard deviation	10,2	%
HorRat-value	**1,1**	
Recovery rate	96	%

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 - 2021***
PT name	***Allergens IV: Celery, Mustard and Sesame in Mayonnaise with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *Mayonnaise / Ingredients: 50% rapeseed oil, water, brandy vinegar, sugar, egg yolk, wheat starch, salt, modified starch, thickener: xanthan gum, guar gum, sodium alginate, acidity regulator: sodium acetate, natural flavor, 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:* *gekühlt 2 - 10 °C (PT period)*
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 July 23rd 2021***
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
		Germany
		SWITZERLAND
		CANADA
		ITALY
		PORTUGAL
		ITALY
		Germany
		Germany
		GREAT BRITAIN
		USA
		SPAIN
		POLAND
		FRANCE
		Germany
		CANADA
		GREAT BRITAIN
		Germany
		Germany
		FRANCE
		GREAT BRITAIN
		GREAT BRITAIN
		USA
		VIETNAM
		SPAIN
		Germany
		CANADA
		GREECE

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

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

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