Proficiency Tests
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Evaluation Report
proficiency test

DLA 04/2017

Allergens IV:

Celery, Mustard and Sesame

in Sausage

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

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Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material was a sausage meat. The basic composition of both sample A and sample B was the same (see table 1). The ingredients were processed in a 9L-cutter in the total sausage meat procedure.
After crushing and homogenization of the basic mixture the spiked sample B was produced as follows:
The spiking material containing the allergenic ingredients celery, mustard and sesame, was prior admixed to potato flour, and then added to the basic mixture and the mixture was homogenized.
Prior to use the allergen premix was sieved by means of a centrifugal mill (mesh 250 µm).
The samples A and B were portioned after homogenization to approximately 25 g in plastic bottles with screw cap and heated in boiling water-bath for 1 h at ≥ 95°C.

For the spiking level sample, the allergenic compounds celery, mustard and sesame (allergen premix, sieved, see above) were added during a multi-stage addition of potato flour (sieved, mesh 500 µm) and homogenization. Afterwards the whole sample was portioned to approximately 15 g into metallised PET film bags.

The composition of the PT samples and the spiking level sample is given in table 1.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Sausage meat Ingredients: Minced meat (beef/pork) 75%, water 13% / ice 12%, salt 0,34%, sodium citrate 0,38%	100 g/100g	93,3 g/100 g	-
Potato flour Ingredients: Potato, E471, E304, E223, E100	-	5,73 g/100 g	99,7 g/100 g
Celery seed: - as Celery seed powder* - *thereof 20,0% total protein***	-	 232 mg/kg 46,4 mg/kg	 60,5 mg/kg 12,1 mg/kg
Mustard, yellow (Sinapis alba): - as Mustard seed powder* - *thereof 30,6% total protein***	-	 191 mg/kg 58,4 mg/kg	 50,0 mg/kg 15,3 mg/kg
Sesame, white: - as Sesame seed* - *thereof 23,3% total protein***	-	 181 mg/kg 42,2 mg/kg	 47,4 mg/kg 11,0 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	< 0,8 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 material (total nitrogen according to Kjeldahl with F=6,25)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
Because only powdered samples can be analysed be the applied microtracer method, only the spiking level sample was measured. The microtracer analysis of the present PT sample showed a probability of 52%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat value of 1,1 respectively. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis. The sample weights were made with a deviation of ± 10% from recommended sample weight of the test kit instructions and not communicated to the laboratories. After transmission of analysis results by the laboratories, the valid results were calculated on the basis of the exact weightings by DLA and the statistical calculation was carried out according to ISO 13528:2009 Annex B.

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 not fulfilled for the tested sample B by two ELISA methods for sesame ("other ELISA, AgraQuant Plus). The standard deviations Ss were 27,6% and 24,5%, respectively. In contrast, the standard deviations within the sub-samples Sw were low in the ELISA tests with 4,9% and 11% (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [16, 17, 20, 21].

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].

The target standard deviation for allergen PTs used by DLA is 25%, because of the increased heterogeneity standard deviation Ss the evaluation of the participants results for sesame was done considering the standard uncertainty (see 3.6 and 3.8).

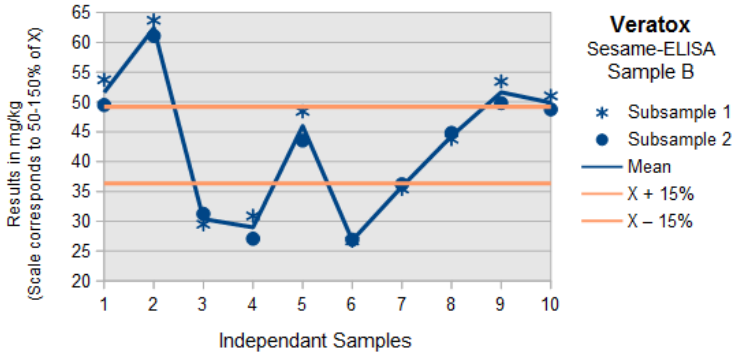
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Neogen Veratox ELISA Sesame

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Sesame 42,8 ± 8,6 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	53,73	49,49	51,61
2	63,69	61,08	62,38
3	29,50	31,24	30,37
4	30,90	27,08	28,99
5	48,43	43,53	45,98
6	26,71	26,92	26,81
7	35,41	36,21	35,81
8	43,76	44,83	44,29
9	53,44	49,81	51,63
10	51,02	48,76	49,89

General average X	42,8	
SD of sample means Sx	11,8	27,6%
SD within-samples Sw	2,08	4,86%
SD between-samples Ss	8,59	20,1%

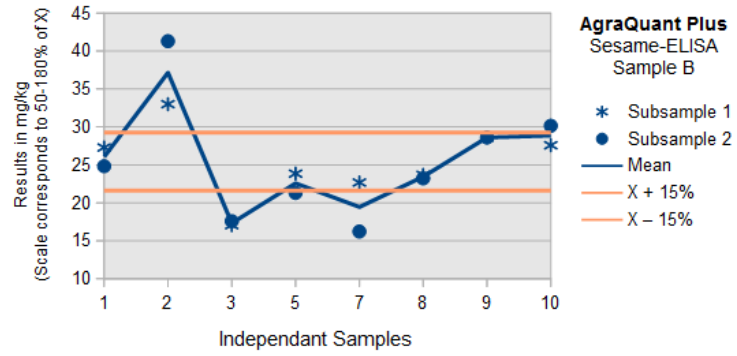


Romerlabs AgraQuant Plus Sesame

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sesame 25,4 ± 5,3 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	27,3	24,8	26,1
2	33,0	41,3	37,1
3	17,1	17,6	17,3
5	23,9	21,3	22,6
7	22,7	16,2	19,5
8	23,7	23,2	23,5
9	28,6	28,6	28,6
10	27,6	30,2	28,9

General average X	25,4	
SD of sample means Sx	6,24	24,5%
SD within-samples Sw	2,85	11,2%
SD between-samples Ss	5,26	20,7%



2.1.2 Stability

The sample material is a sausage meat, which was heated to ≥ 95°C for 1h after production and bottling. The storage stability and shelf life of the samples (microbiological spoilage) was given during the analysis period under indicated storage conditions as shown by prior experiences.

2.2 Sample shipment and information to the test

The portions of test material (sample A and sample B as well as the spiking level sample) were sent to every participating laboratory in the 25th week of 2017. The testing method was optional. The tests should be finished at August 18th 2017 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters celery, mustard and/or sesame in the range of mg/kg in the matrix of sausage.
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.*
The total amounts of samples A and B should homogenized separately before the analysis, because fat and water can separate during the manufacture of the sausage meat.

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. 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.
During evaluation DLA eventually requests detailed information by email on the type of indicated quantitative results from participants concerned.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 30 participants submitted their results in time.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [23, 24, 25, 26]. It is for this reason that we contrast the results of the present proficiency test with several assigned values. Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.

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

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

3.1 Consensus value from participants (assigned value)

The robust mean of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. 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 identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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 3a (ELISA) and table 3b (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} [28-29]

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 11 – 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 [22]. 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 [22].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [25]. 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} [30-34]

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 [20], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [17-19], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [21] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [16].

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

Table 4: ELISA-Validation

Literature [16-22]	**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 5: PCR-Validation

Literature [16]	**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. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and 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) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty of the assigned value

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

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

If $U_{(x_{pt})} \leq 0{,}3\ \sigma_{pt}$ 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 Quotient $U(x_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

3.9 Figures

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

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of allergen-ELISAs proposed by the AOAC was used [21]. For quantitative PCR determinations we use the same range of acceptance.

4. Results

Summarized overview on results:

The detection and determination of the allergens celery, mustard and sesame in the present PT sample matrix of sausage (semi-preserve, heated ≥ 95°C, 1 h) and in the spiking level sample (without processing) by ELISA and PCR methods can be summarized as follows:

- *ELISA methods were applied for the determination of mustard and sesame.*
- *Mustard with a content of approx. 190 mg/kg in the sausage meat sample B was not detected by 81% of participant's results using ELISA methods. The few positive results were below the LOQ of the tests with one or two exceptions.*
- *On the other hand, in the spiking level sample mustard was detected by all ELISA methods, except for a single-result of one method, with recovery rates higher than 100% (max. 250%).*
- *Sesame with a content of approx. 180 mg/kg in the sausage meat sample B was detectable and quantitatively measurable by 3 of the applied ELISA methods (and by 2 methods with one individual result each). Sesame could not be detected by two other ELISA methods. The amount of quantitative results was strongly method-dependent. The recovery rates were all below 50%, with one exception (1-54%).*
- *In the spiking level sample sesame could be determined by all applied ELISA methods. The method-dependent response of quantitative results was found for the spiking level too. Thus the robust mean for one method was about 5,8 mg/kg and for another method about 160 mg/kg at recovery rates of 12% and 340%, respectively.*

- *PCR methods were applied for the detection of celery, mustard and sesame.*
- *For all three allergens 95% to 100% qualitatively positive results were obtained for the sausage meat sample B. The few submitted quantitative results showed a higher variation. In the cases of mustard and sesame for both sample B and the spiking level sample. A statistical evaluation was therefore made only for celery in the spiking level sample. The recovery rate was 28%.*

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).

For celery all present results were submitted as celery (seed), thus no recalculation was necessary.

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

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

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

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

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

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

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		
7	negative		positive		2/2 (100%)	ASU	
12	negative		positive		2/2 (100%)	ASU	
16	negative		positive		2/2 (100%)	ASU	
8b	negative		positive		2/2 (100%)	MS	
26	negative		positive		2/2 (100%)	MS	
30a	negative	-	positive	-	2/2 (100%)	SFA-4p	
3	negative	<1	positive	121	2/2 (100%)	SFA-ID	
8a	negative		positive	61,0	2/2 (100%)	SFA-ID	
9	negative		positive		2/2 (100%)	SFA-ID	
14	negative		positive		2/2 (100%)	SFA-ID	
15	negative		positive		2/2 (100%)	SFA-ID	
20	negative		positive		2/2 (100%)	SFA-ID	
25	negative	<0.4	positive	>0.4	2/2 (100%)	SFA-ID	
27a	negative		positive		2/2 (100%)	SFA-ID	
18	negative	<0,4	positive	20,3	2/2 (100%)	SFA-Q	
27b	negative	< 1,0	positive	5,78	2/2 (100%)	SFA-Q	
30b	negative	-	positive	5,00	2/2 (100%)	SFA-Q	
6	negative		negative		1/2 (50%)	div	
13	negative	-	positive	55,0	2/2 (100%)	div	
19	negative	n.n.	positive	130	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		19	
Number negative	20		1	
Percent positive	0		95	
Percent negative	100		5	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
MS = Microsynth
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B. One negative result for sample B was obtained with a method not specified.

Quantitative Valuation PCR: Sample B

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

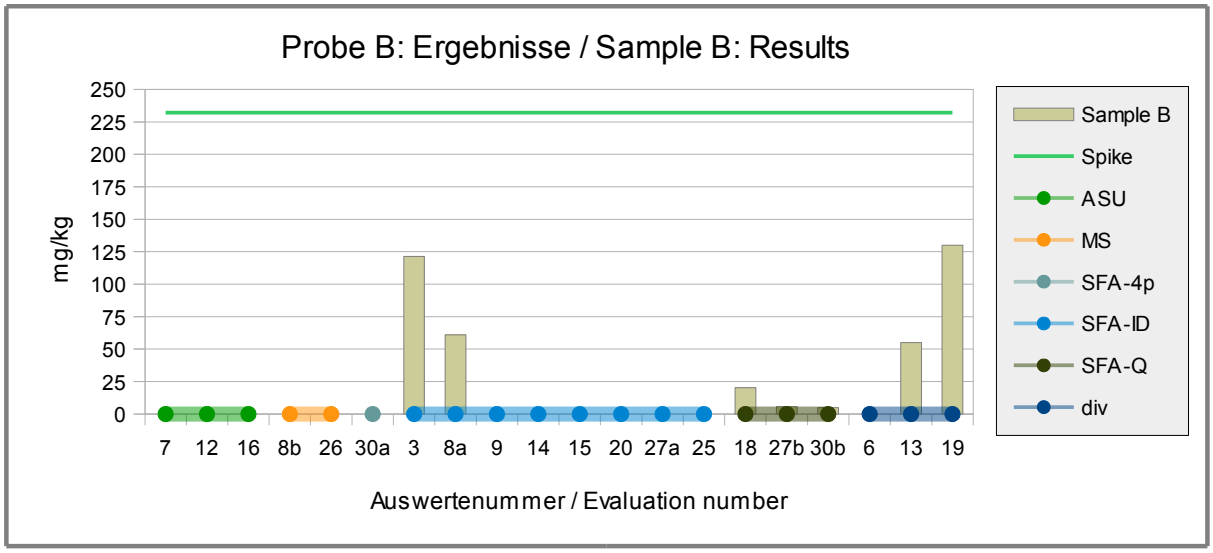


Abb./Fig. 1: PCR Results Celery
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

Evaluation number	Celery	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
7			ASU	
12			ASU	
16			ASU	
8b			MS	
26			MS	
30a	-		SFA-4p	
3	119		SFA-ID	outlier X_{all}, excluded
8a	24,0	1,7	SFA-ID	
9			SFA-ID	
14			SFA-ID	
15			SFA-ID	
20			SFA-ID	
25	>0.4		SFA-ID	
27a			SFA-ID	
18	12,9	-1,0	SFA-Q	
27b	14,5	-0,6	SFA-Q	
30b	13,2	-0,9	SFA-Q	
6			div	
13	20,0	0,7	div	
19			div	

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

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

div = not indicated / other method

Comments:

Due to <8 results no kernel density estimation was done.

Characteristics: Quantitative Evaluation PCR Celery

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5 *
Number of outliers	0
Mean	16,9
Median	14,5
Robust Mean (X)	**16,9**
Robust standard deviation (S*)	**5,55**
Target range:	
Target standard deviation σ_{pt}	**4,23**
lower limit of target range	**8,46**
upper limit of target range	**25,4**
Quotient S/σ_{pt}*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*3,10*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,73*
Results in the target range	*5*
Percent in the target range	*100*

** result evaluation no. 3 excluded*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a normal variability of results. The quotient S/σ_{pt} was 1,3. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited, because there are only a few results.*

The robust mean of the evaluation was 28% of the spiking level to the spiking level sample and thus below the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Celery").

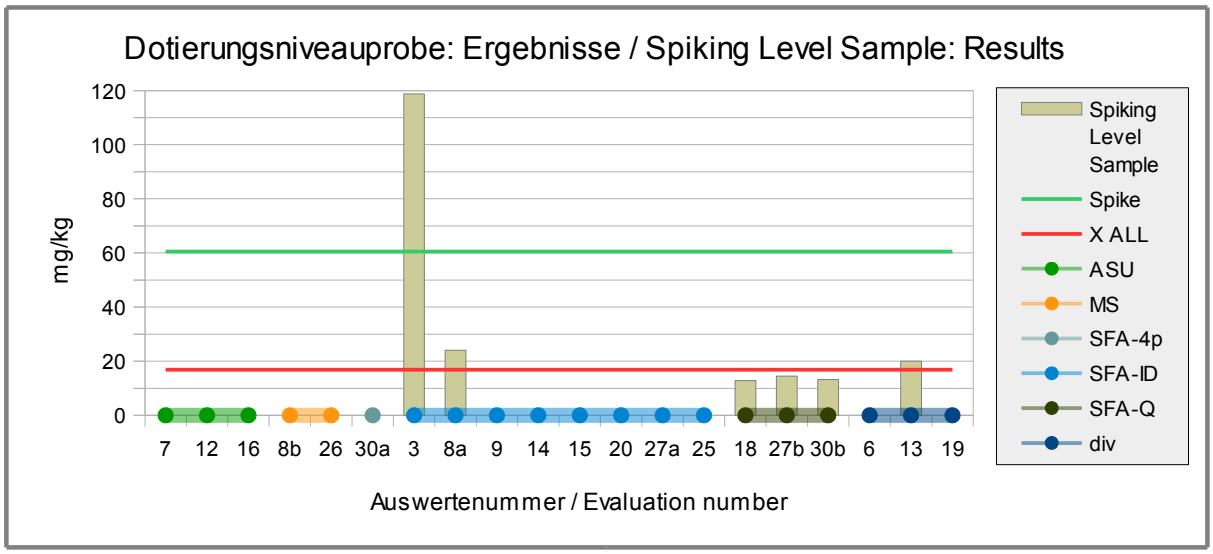


Abb./Fig. 2: PCR Results Celery
green line = Spiking level
red line = Assigned value robust mean all included results
round symbols = Applied methods (see legend)

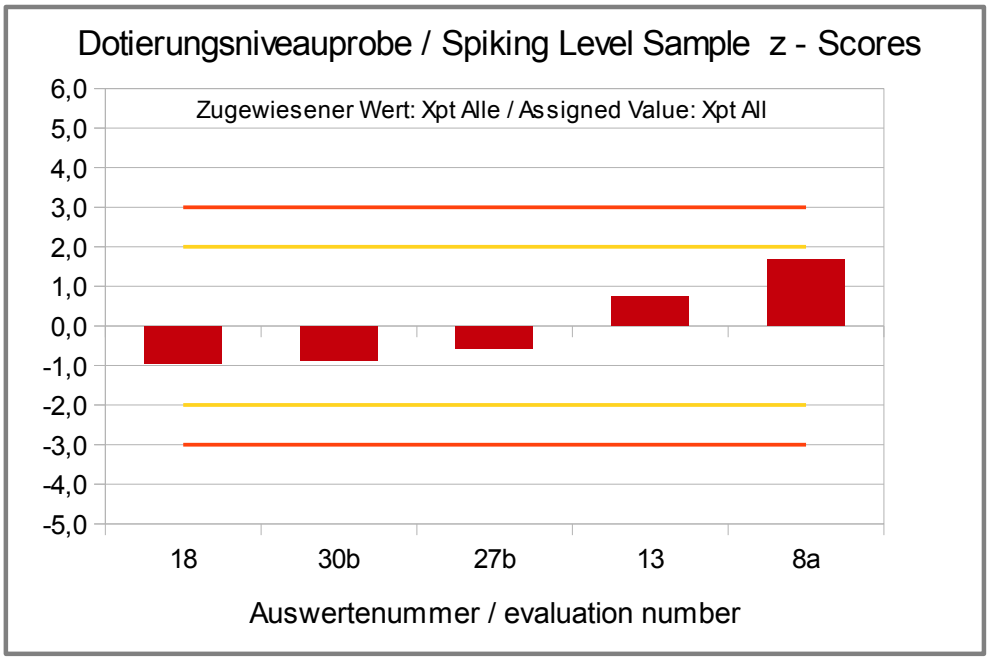


Abb./Fig. 3:
z-Scores (PCR Results Celery)
Assigned value robust mean of all results

Recovery Rates PCR for Celery:
Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
7					ASU	
12					ASU	
16					ASU	
8b					MS	
26					MS	
30a	-		-		SFA-4p	
3	119	196	121	**52**	SFA-ID	
8a	24,0	40	61,0	26	SFA-ID	
9					SFA-ID	
14					SFA-ID	
15					SFA-ID	
20					SFA-ID	
25	>0.4		>0.4		SFA-ID	
27a					SFA-ID	
18	12,9	21	20,3	8,7	SFA-Q	
27b	14,5	24	5,78	2,5	SFA-Q	
30b	13,2	22	5,00	2,2	SFA-Q	
6					div	
13	20,0	33	55,0	24	div	
19			130	**56**	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**2**
Percent in RA	**0**	Percent in RA	**29**

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

** Range of acceptance of AOAC for allergen ELISAS

Methodes:
ASU = ASU §64 Methode/method
MS = Microsynth
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the spiking material sample no recovery rate within the range of the AOAC-recommendation of 50-150% was obtained. For the processed spiked food matrix sample B 29% (2) of the recovery rates were within the recommended range.

4.2 Proficiency Test Mustard

4.2.1 ELISA Results: Mustard (Sinapis alba)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
6	negative	<2	negative	<2	2/2 (100%)	AQ	
18	negative	<1	negative	<1	2/2 (100%)	AQ	
28	negative	<2,0	negative	<2,0	2/2 (100%)	AQ	
23	negative	<2	negative	<2	2/2 (100%)	BC	
25	negative	<3,27	negative	<3,27	2/2 (100%)	ES	result converted °
1	negative	<1	positive	2,02	1/2 (50%)	IL	
4	negative		positive	<0,5	1/2 (50%)	NL	
2	negative		negative		2/2 (100%)	RS-F	
3	negative	<0,5	negative	<0,5	2/2 (100%)	RS-F	
7	positive	2,21	negative	<0,5	1/2 (50%)	RS-F	samples mixed up?
9	negative		negative		2/2 (100%)	RS-F	
13	negative	-	positive	<0,5	1/2 (50%)	RS-F	
16	negative		positive		1/2 (50%)	RS-F	
17	negative	<0,5	negative	<0,5	2/2 (100%)	RS-F	
19	negative	<0,5	negative	<0,5	2/2 (100%)	RS-F	
22	negative	<0,5	negative	<0,5	2/2 (100%)	RS-F	
27	negative	<0,5	negative	<0,5	2/2 (100%)	RS-F	
10	negative		negative		2/2 (100%)	VT	
11	negative	<8,17	negative	<8,17	2/2 (100%)	VT	
21	negative		negative		2/2 (100%)	VT	
29	negative	<2,5	negative	<2,5	2/2 (100%)	VT	

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	Sample A		Sample B	
Number positive	1		4	
Number negative	20		17	
Percent positive	5		19	
Percent negative	95		81	
Consensus value	negative		negative	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

The consensus values are not in qualitative agreement with the spiking of sample B, for which only 4 positive results were obtained (methods IL, NL and RS-F). There was one positive result submitted for sample A, while sample B was indicated negative at the same time.

Quantitative Valuation ELISA: Sample B

Since there was only one quantitative result available, no figure is presented and no evaluation was done.

Quantitative Valuation ELISA: Spiking Level Sample

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
6	127	2,3		AQ	
18	>60			AQ	
28	85,6	0,24		AQ	
23	111	1,5		BC	
25	52,3	-1,4		ES	result converted °
1	95,1	0,71		IL	
4	20,8	-3,0		NL	
2				RS-F	
3	75,6	-0,25	0,03	RS-F	
7	76,3	-0,22	0,07	RS-F	
9				RS-F	
13	60,0	-1,0	-0,80	RS-F	
16				RS-F	
17	>13,5			RS-F	
19	78,0	-0,13	0,16	RS-F	
22	76,0	-0,23	0,05	RS-F	
27	73,5	-0,36	-0,08	RS-F	
10	112	1,6		VT	
11				VT	
21				VT	
29	73,3	-0,37		VT	

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Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

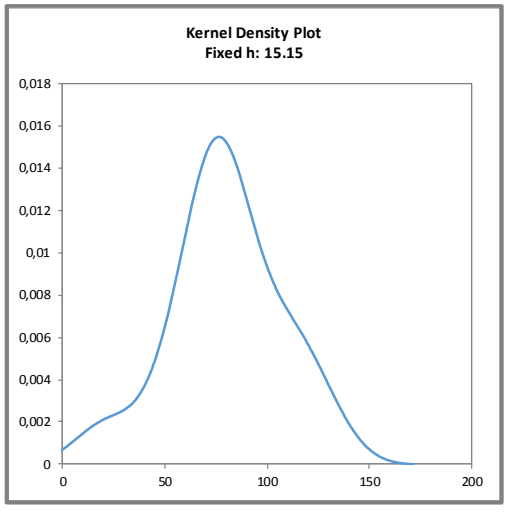

Abb. / Fig. 4:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a normal distribution of results with slight shoulders below 50 mg/kg and above 100 mg/kg, due to single results of different methods.

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	14	6
Number of outliers	0	0
Mean	79,7	73,2
Median	76,1	75,8
Robust Mean (X)	**80,7**	**75,0**
Robust standard deviation (S*)	**25,1**	**2,89**
Target range:		
Target standard deviation σ_{pt}	**20,2**	**18,80**
lower limit of target range	**40,4**	**37,5**
upper limit of target range	**121,0**	**113,0**
Quotient S/σ_{pt}*	*1,2*	*0,15*
Standard uncertainty $U_{(Xpt)}$	*8,38*	*1,47*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,42*	*0,08*
Results in the target range	*12*	*6*
Percent in the target range	*86*	*100*

Method:
RS-F= R-Biopharm, Ridascreen® FAST

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences. Both, the evaluation of the results of all methods and the results from method RS-F showed a normal variability of results. The quotients S/σ_{pt} were below 2,0.*
The robust standard deviation is in the range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for the methods.

The robust means of the evaluations were 161% and 150% of the spiking level of mustard to the spiking level sample and thus above and at the upper limit of the recommendations for the applied methods (s. 3.4.3 and "recovery rates for mustard").

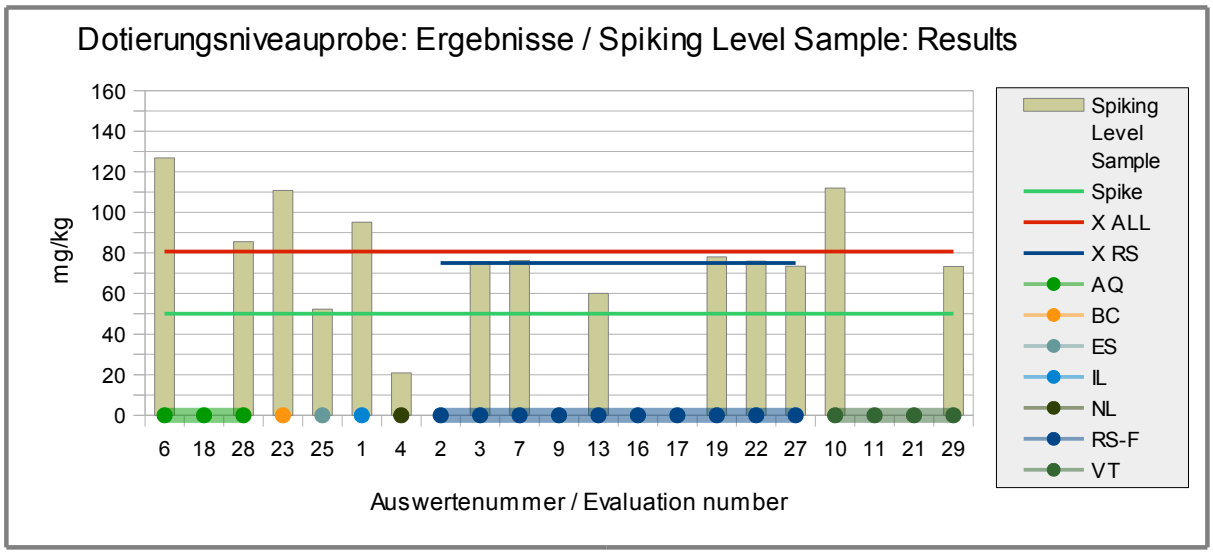

Abb./Fig. 5: ELISA Results Mustard
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

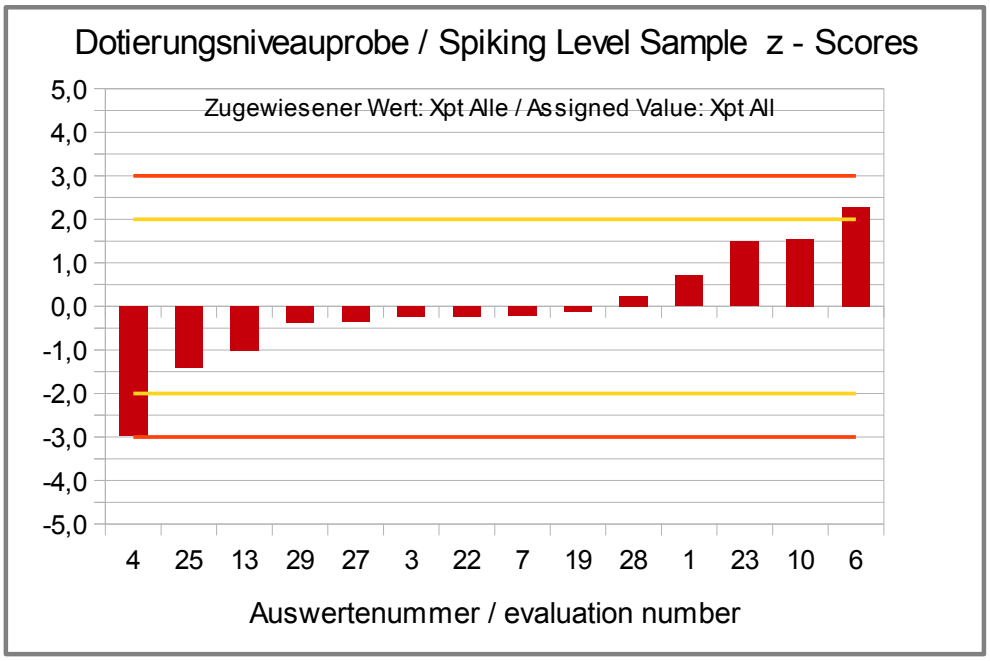

Abb./Fig. 6:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of all results

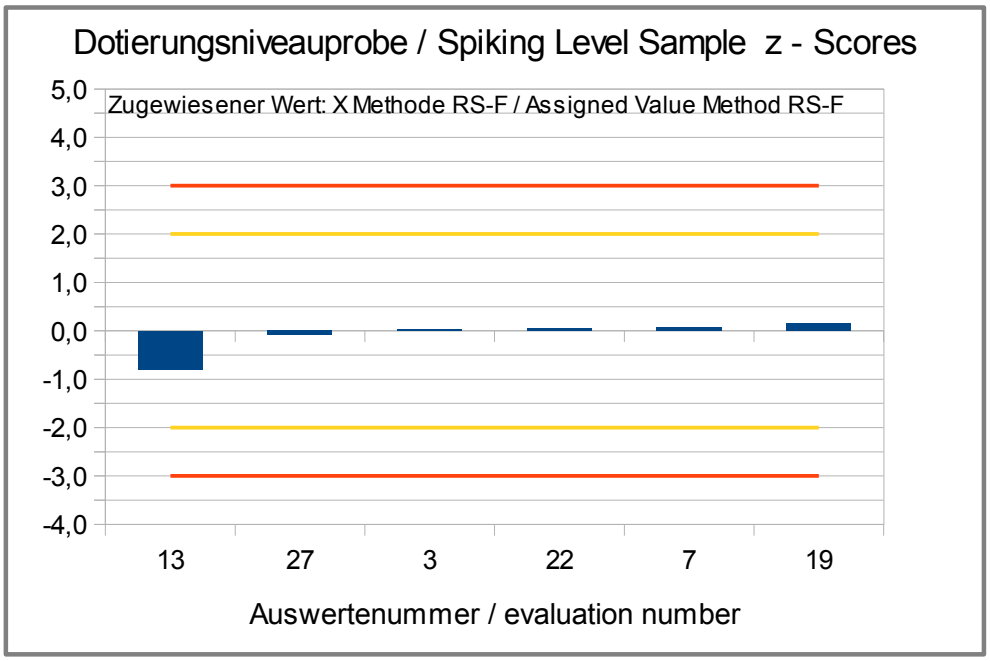

Abb./Fig. 7:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS (R-Biopharm, Ridascreen)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	127	254	<2		AQ	
18	>60		<1		AQ	
28	85,6	171	<2,0		AQ	
23	111	222	<2		BC	
25	52,3	**105**	<3,27		ES	result converted °
1	95,1	190	2,02	1,1	IL	
4	20,8	42	<0,5		NL	
2					RS-F	
3	75,6	151	<0,5		RS-F	
7	76,3	153	<0,5		RS-F	
9					RS-F	
13	60,0	**120**	<0,5		RS-F	
16					RS-F	
17	>13,5		<0,5		RS-F	
19	78,0	156	<0,5		RS-F	
22	76,0	152	<0,5		RS-F	
27	73,5	**147**	<0,5		RS-F	
10	112	224			VT	
11			<8,17		VT	
21					VT	
29	73,3	**147**	<2,5		VT	

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RA**	50-150 %	RA**	50-150 %
Number in RA	**4**	Number in RA	**0**
Percent in RA	**29**	Percent in RA	**0**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking material sample 29% (4) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the processed food matrix sample B only one result was available with a low recovery rate of approx. 1%.

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		
8b	negative		positive		2/2 (100%)	MS	
30a	negative	-	positive	-	2/2 (100%)	SFA-4p	
3	negative	<1	positive	82,9	2/2 (100%)	SFA-ID	
8a	negative		positive	204	2/2 (100%)	SFA-ID	
9	negative		positive		2/2 (100%)	SFA-ID	
14	negative		positive		2/2 (100%)	SFA-ID	
15	negative		positive		2/2 (100%)	SFA-ID	
25	negative	<0,4	positive	>0,4	2/2 (100%)	SFA-ID	
27	negative		positive		2/2 (100%)	SFA-ID	
12	negative		positive		2/2 (100%)	div	
13	negative	-	positive	65,0	2/2 (100%)	div	
16	negative		positive		2/2 (100%)	div	
19	negative	n.n.	positive	230	2/2 (100%)	div	
26	negative		positive		2/2 (100%)	div	
30b	negative	-	positive	3,90	2/2 (100%)	div	

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

Methods:

MS = Microsynth

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

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

Quantitative Valuation PCR: Sample B

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

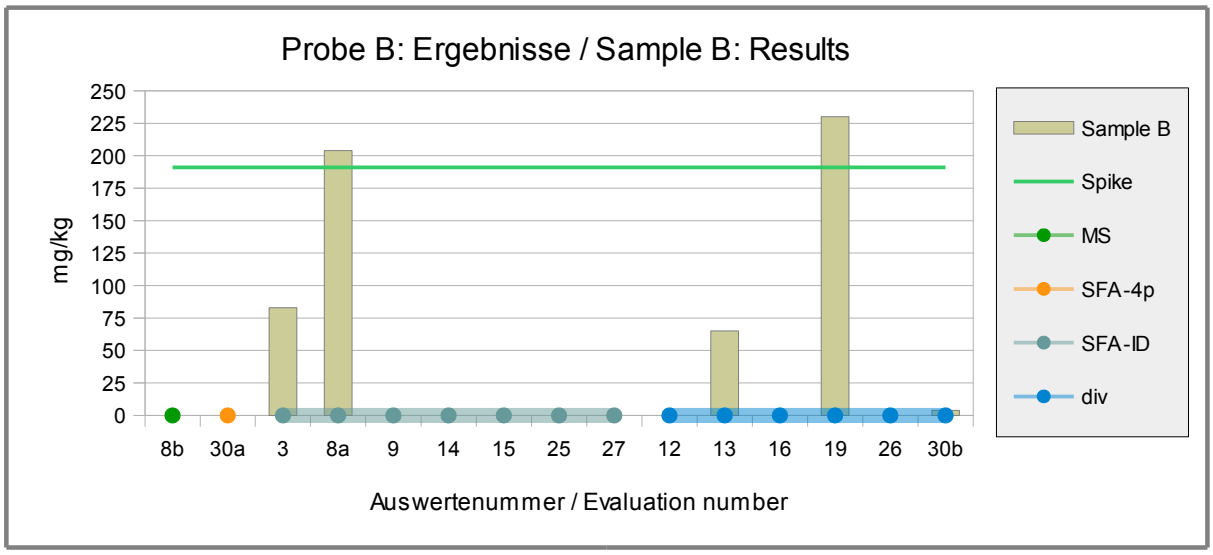

Abb./Fig. 8: PCR Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

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

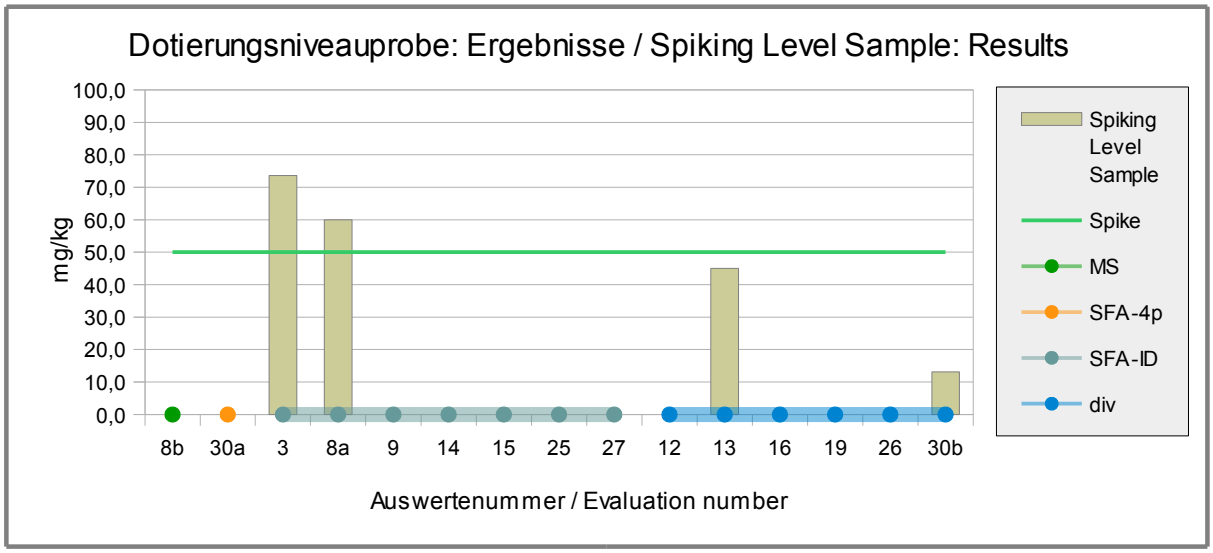

Abb./Fig. 9: PCR Results Mustard
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Mustard:
Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
8b					MS	
30a	-		-		SFA-4p	
3	73,7	**147**	82,9	43	SFA-ID	
8a	60,0	**120**	204	**107**	SFA-ID	
9					SFA-ID	
14					SFA-ID	
15					SFA-ID	
25	>0,4		>0,4		SFA-ID	
27					SFA-ID	
12					div	
13	45,0	**90**	65,0	34	div	
16					div	
19			230	**120**	div	
26					div	
30b	13,1	26	3,90	2,0	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**3**	Number in RA	**2**
Percent in RA	**75**	Percent in RA	**40**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
MS = Microsynth
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the spiking level sample 3 out of 5 (75%) participants obtained a recovery rate by PCR within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B 2 out of 5 (40%) of the obtained recovery rates were within the recommended range.

4.3 Proficiency Test Sesame

4.3.1 ELISA Results: Sesame

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
6	negative	<2	negative	<2	1/2 (50%)	AQ	
18	negative	<0,2	positive	1,20	2/2 (100%)	AQ	
23	negative	<2	negative	<2	1/2 (50%)	BC	
4	negative		positive	76,5	2/2 (100%)	BK	
10	negative		positive	48,5	2/2 (100%)	BK	
5	negative	<2,16	negative	<2,16	1/2 (50%)	ES	result converted °
9	negative		negative		1/2 (50%)	ES	
11	negative	<0,5	negative	<0,5	1/2 (50%)	ES	
17	negative	<2,16	negative	<2,16	1/2 (50%)	ES	result converted °
21	negative		negative		1/2 (50%)	ES	
25	negative	<2,16	negative	<2,16	1/2 (50%)	ES	result converted °
28	negative	<2,16	negative	<2,16	1/2 (50%)	ES	result converted °
29	negative	<2,16	negative	<2,16	1/2 (50%)	ES	result converted °
1	negative	<0,2	positive	3,78	2/2 (100%)	IL	
2	negative		positive	10,3	2/2 (100%)	RS-F	
3	negative	<2,5	positive	19,1	2/2 (100%)	RS-F	outlier X_{R-F}
7	negative	<2,5	positive	7,76	2/2 (100%)	RS-F	
13	negative	-	positive	10,0	2/2 (100%)	RS-F	
19	negative		positive	10,0	2/2 (100%)	RS-F	
22	negative	<2,5	positive	8,50	2/2 (100%)	RS-F	
11	negative	<2,5	positive	98,0	2/2 (100%)	VT	result converted °, outlier X_{all}
24	negative	<6,25	positive	38,0	2/2 (100%)	VT	

° calculation p. 19

	Sample A		Sample B	
Number positive	0		12	
Number negative	22		10	
Percent positive	0		55	
Percent negative	100		45	
Consensus value	negative		none*	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

** the qualitative valuation was done with respect to the spiking, because no consensus value could be established for the different methods*

Comments:

The consensus values are in qualitative agreement with sample A. For the spiked sample B positive results were obtained by the methods BK, IL, RS-F and VT. While method AQ gave one positive and one negative result.

Quantitative Valuation ELISA: Sample B

Evaluation number	Sesame	z-Score Xpt_{ALL}	z'-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
6	<2			AQ	
18	1,20			AQ	
23	<2			BC	
4	76,5			BK	
10	48,5			BK	
5	<2,16			ES	result converted °
9				ES	
11	<0,5			ES	
17	<2,16			ES	result converted °
21				ES	
25	<2,16			ES	result converted °
28	<2,16			ES	result converted °
29	<2,16			ES	result converted °
1	3,78			IL	
2	10,3		0,15	RS-F	
3	19,1		3,4	RS-F	outlier $X_{R\text{-}F}$
7	7,76		-0,80	RS-F	
13	10,0		0,03	RS-F	
19	10,0		0,03	RS-F	
22	8,50		-0,53	RS-F	
11	98,0			VT	result converted °, outlier X_{all}
24	38,0			VT	

° calculation p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

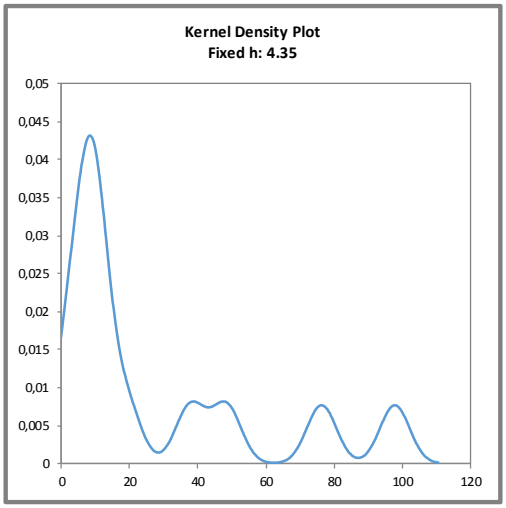

Abb. / Fig. 10:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a main peak at approx. 10 mg/kg, due to method RS-F results. Further side peaks are caused by other methods with only a few number of results.

Characteristics: Quantitative Evaluation ELISA Sesame

Sample B

Statistic Data	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	6
Number of outliers	1
Mean	10,9
Median	10,0
Robust Mean (X)	**9,91**
Robust standard deviation (S*)	**2,01**
Target range:	
Target standard deviation $\sigma_{pt'}$	**2,68**
lower limit of target range	**4,55**
upper limit of target range	**15,3**
Quotient S/$\sigma_{pt'}$*	*0,75*
Standard uncertainty $U_{(Xpt)}$	*1,03*
Quotient $U_{(Xpt)}/\sigma_{pt'}$	*0,38*
Results in the target range	*5*
Percent in the target range	*83*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed clear method-dependent differences of the applied methods. Therefore a joined evaluation of all results was not done.

The evaluation of the results of method RS-F showed a normal to low variability of results. The robust standard deviation is in the range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. However, because of the afore determined value of the heterogeneity standard deviation Ss of >15% (see 2.1.1 Homogeneity) the target standard deviation was expanded considering the standard uncertainty and evaluating by z'-scores (s. 3.6 and 3.8).

The robust mean of the evaluation of method RS-F results was 5,5% of the spiking level of sesame to sample B and thus clearly below the recommendations for the applied methods (s. 3.4.3 and "recovery rates for sesame").

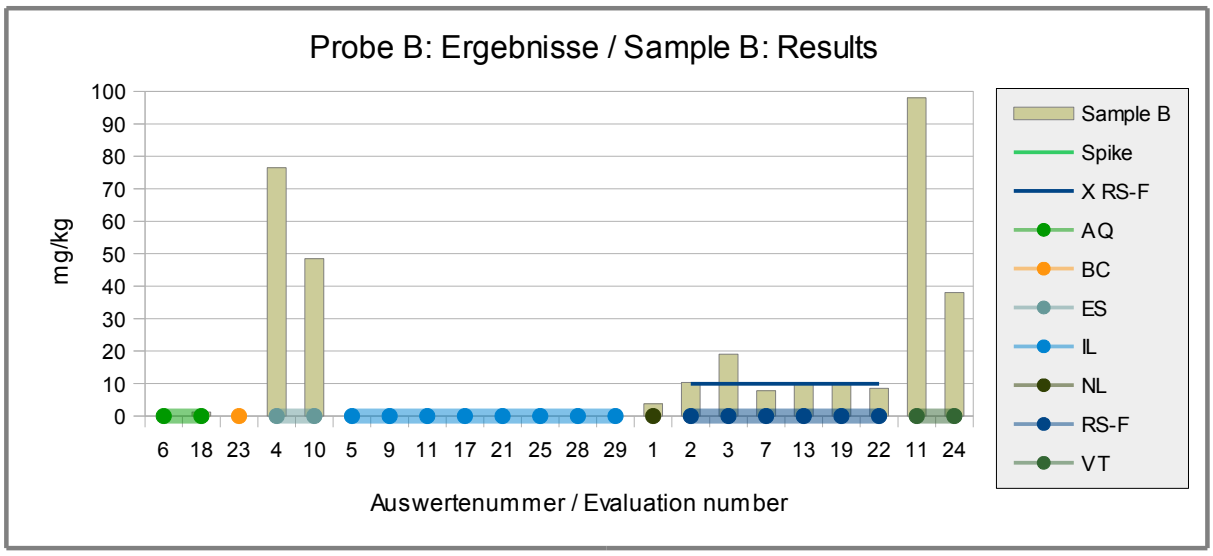

Abb./Fig. 11: ELISA Results Sesame
(Spiking level (Spike) of 181 mg/kg not shown)
blue line = Assigned value robust mean results method ES
round symbols = Applied methods (see legend)

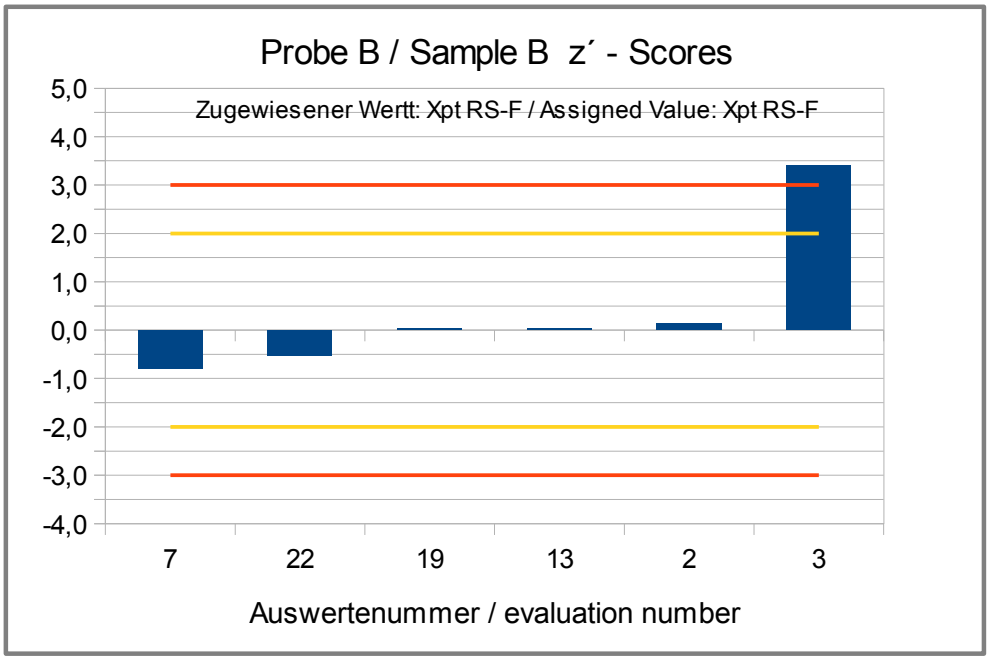

Abb./Fig. 12:
z'-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative Valuation ELISA: Spiking Level Sample

Comments:
The kernel density estimation (Fig. 13) and the figure of results (Fig. 14) showed an obvious method-dependent distribution of results. Therefore a joined evaluation of all results was not done.
Statistical evaluation was made for methods with ≥ 5 results of the single methods (ES, RS-F).

Evaluation number	Sesame	z-Score Xpt_{ALL}	z-Score Xpt_{ES}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]					
6	39,8				AQ	
18	60,0				AQ	
23	34,6				BC	
4	330				BK	
10	198				BK	
5	7,07		0,90		ES	result converted °
9					ES	
11					ES	
17	5,60		-0,12		ES	result converted °
21					ES	
25	8,19		1,7		ES	result converted °
28	3,23		-1,8		ES	result converted °
29	4,74		-0,71		ES	result converted °
1	50,6				IL	
2					RS-F	
3	210			1,1	RS-F	
7	197			0,82	RS-F	
13	130			-0,82	RS-F	
19	140			-0,57	RS-F	
22	140			-0,57	RS-F	
11					VT	
24	349				VT	

° calculation p. 19

Methodes:
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

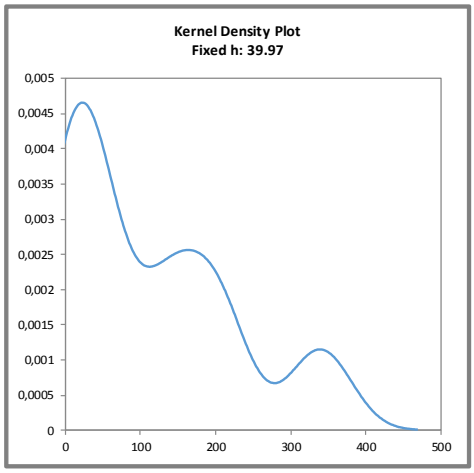

Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a main peak at approx. 5-6 mg/kg (method ES) and a second peak at approx. 160 mg/kg (method RS-F). These peaks are overlaying the few results of other methods. A further side peak >300 mg/kg is caused by a single result (VT).

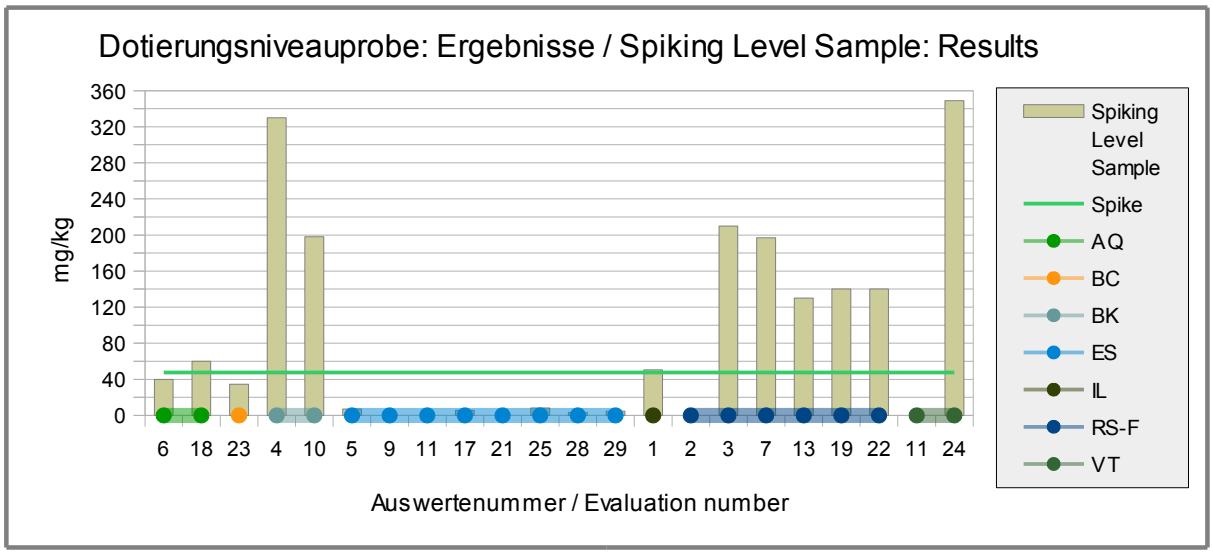

Abb./Fig. 14: ELISA Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Characteristics: Quantitative Evaluation ELISA Sesame

Spiking Level Sample

Statistic Data	**Method ES** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ES}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	5	5
Number of outliers	0	0
Mean	5,77	163
Median	5,60	140
Robust Mean (X)	**5,77**	**163**
Robust standard deviation (S*)	**2,20**	**42,1**
Target range:		
Target standard deviation σ_{pt}	**1,44**	**40,8**
lower limit of target range	**2,88**	**81,7**
upper limit of target range	**8,65**	**245**
Quotient S/σ_{pt}*	*1,5*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*1,23*	*23,5*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,85*	*0,58*
Results in the target range	*5*	*5*
Percent in the target range	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed clear method-dependent differences of the applied methods. Therefore a joined evaluation of all results was not done.

Both, the evaluation of the results of method ES and the results from method RS-F showed a normal variability of results. The quotients S/σ_{pt} were below 2,0.*
The robust standard deviation is in the range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given.

The robust means of the evaluations were 12% (ES) and 341% (RS-F) of the spiking level of sesame to the spiking level sample and thus below and above the limits of the recommendations for the applied methods, respectively (s. 3.4.3 and "recovery rates for sesame").

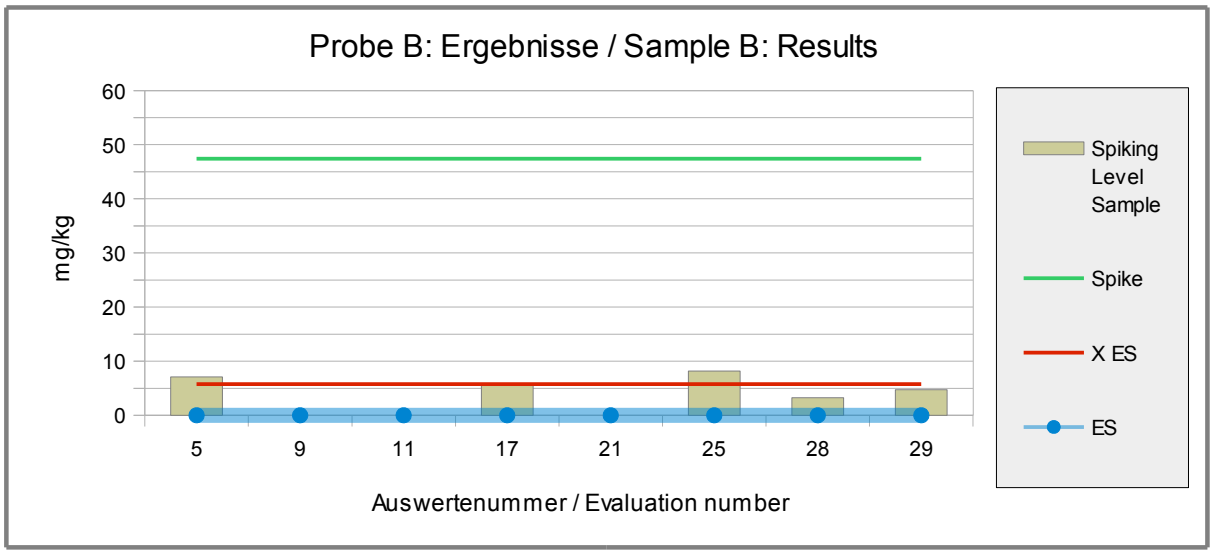

Abb./Fig. 15: ELISA Results Sesame Method ES
green line = Spiking level
red line = Assigned value robust mean results method ES
round symbols = Applied methods (see legend)

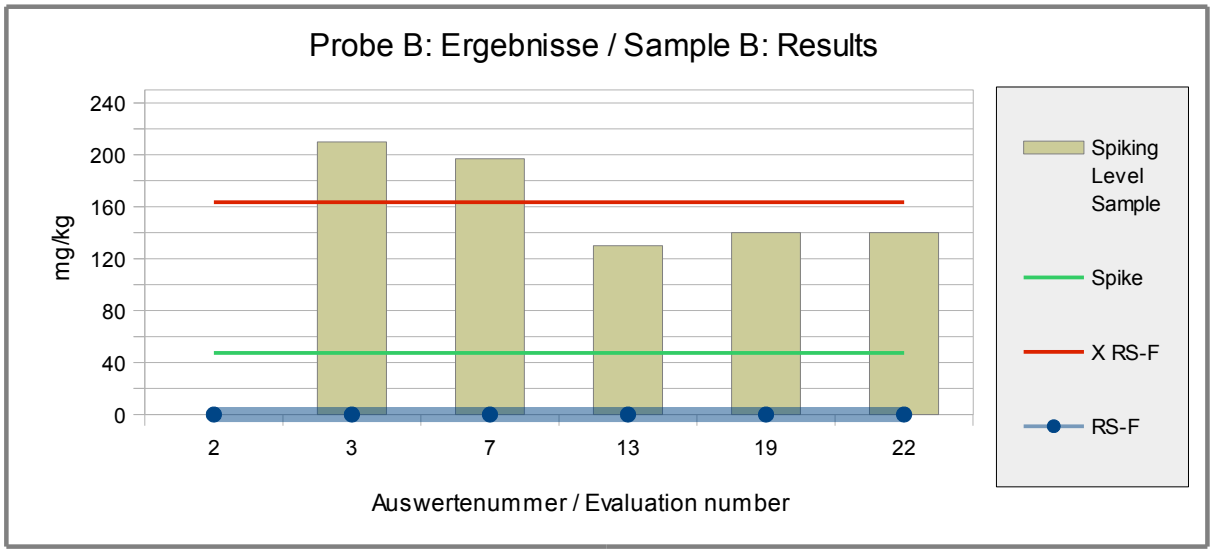

Abb./Fig. 16: ELISA Results Sesame Method RS-F
green line = Spiking level
red line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

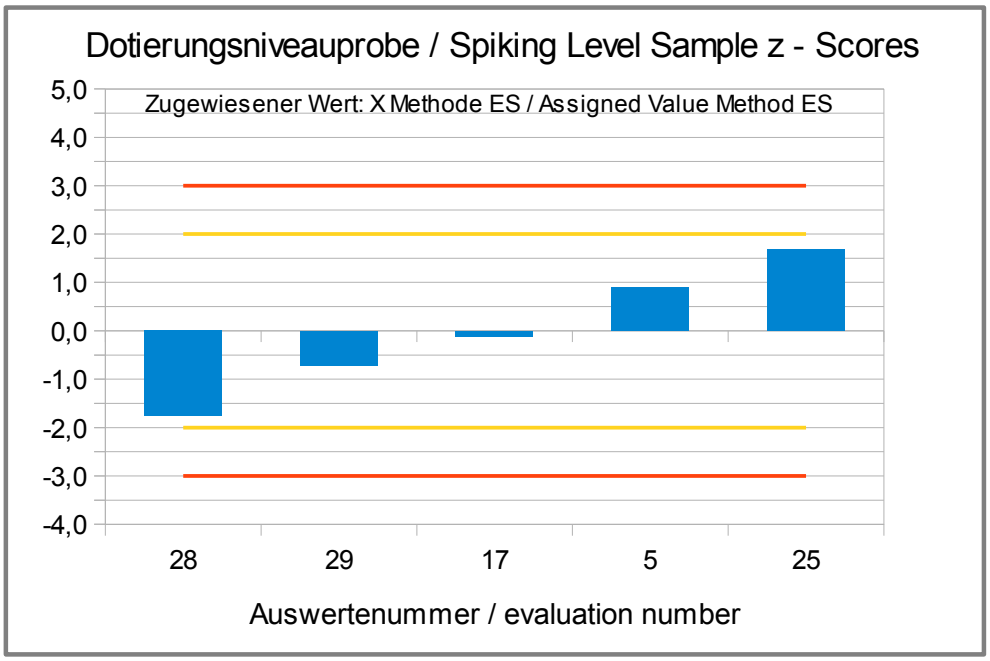

Abb./Fig. 17:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method ES (ELISA-Systems)

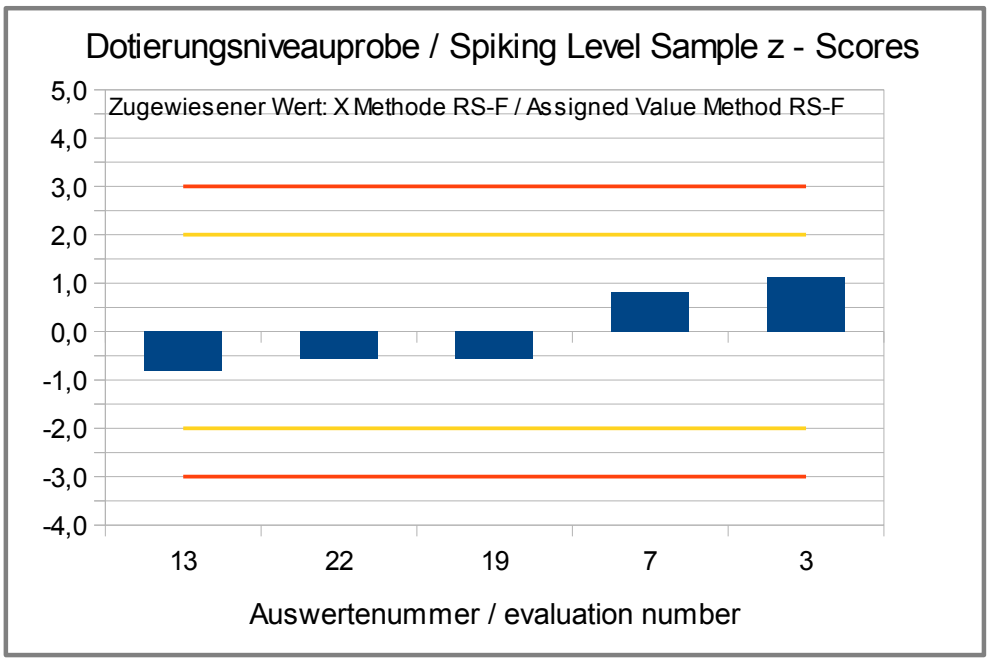

Abb./Fig. 18:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates ELISA for Sesame:
Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	39,8	**84**	<2		AQ	
18	60,0	**127**	1,20	0,7	AQ	
23	34,6	**73**	<2		BC	
4	330	696	76,5	42	BK	
10	198	418	48,5	27	BK	
5	7,07	15	<2,16		ES	result converted °
9					ES	
11			<0,5		ES	
17	5,60	12	<2,16		ES	result converted °
21					ES	
25	8,19	17	<2,16		ES	result converted °
28	3,23	7	<2,16		ES	result converted °
29	4,74	10	<2,16		ES	result converted °
1	50,6	**107**	3,78	2,1	IL	
2			10,3	5,7	RS-F	
3	210	443	19,1	11	RS-F	
7	197	416	7,76	4,3	RS-F	
13	130	274	10,0	5,5	RS-F	
19	140	295	10,0	5,5	RS-F	
22	140	295	8,50	4,7	RS-F	
11			98,0	**54**	VT	
24	349	736	38,0	21	VT	

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**4**	Number in RA	**1**
Percent in RA	**24**	Percent in RA	**8**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking level sample 4 participants obtained recovery rates by the ELISA methods AQ, BC and IL within the range of the AOAC-recommendation of 50-150%. The recovery rates obtained by method ES were below and the recovery rates obtained by the methods BK, RS-F and VT were above the range of acceptance.
For the processed food matrix sample B one recovery rate was in the range of acceptance.

4.3.2 PCR Results: Sesame

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
8b	negative		positive		2/2 (100%)	MS	
26	negative		positive		2/2 (100%)	MS	
8a	negative		positive	58,5	2/2 (100%)	SFA-ID	
9	negative		positive		2/2 (100%)	SFA-ID	
14	negative		positive		2/2 (100%)	SFA-ID	
15	negative		positive		2/2 (100%)	SFA-ID	
16	negative		positive		2/2 (100%)	SFA-ID	
25	negative	<0,4	positive	>0,4	2/2 (100%)	SFA-ID	
27	negative		positive		2/2 (100%)	SFA-ID	
30	negative	-	positive	4	2/2 (100%)	SFA-Q	
7	negative		positive		2/2 (100%)	div	
12	negative		positive		2/2 (100%)	div	
13	negative	-	positive	145	2/2 (100%)	div	
19	negative	n.n.	positive	92	2/2 (100%)	div	

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

Methods:
MS = Microsynth
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

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

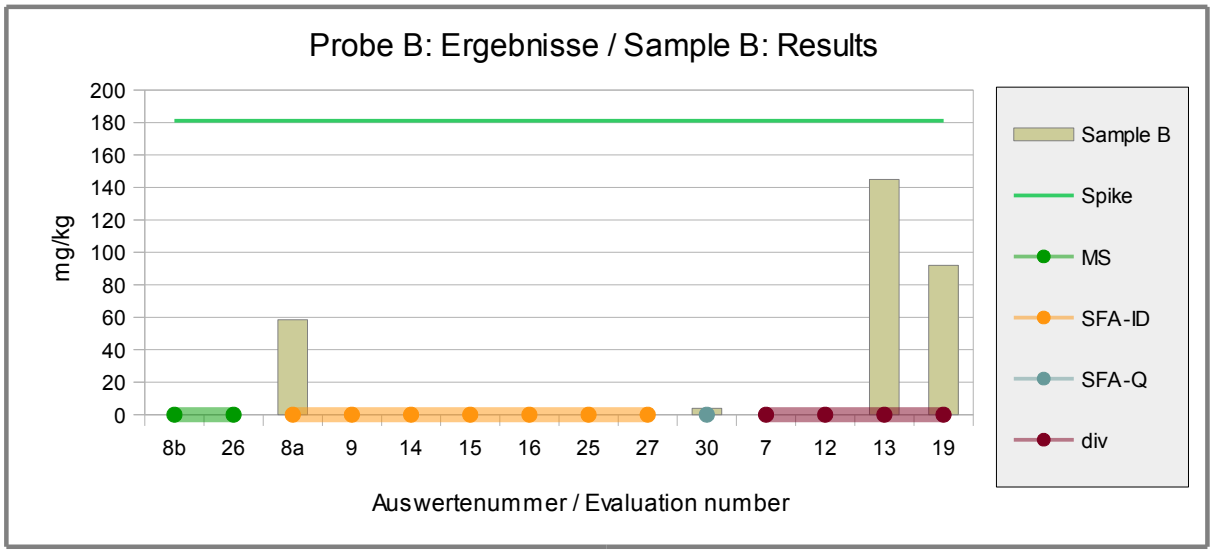

Abb./Fig. 19: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

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

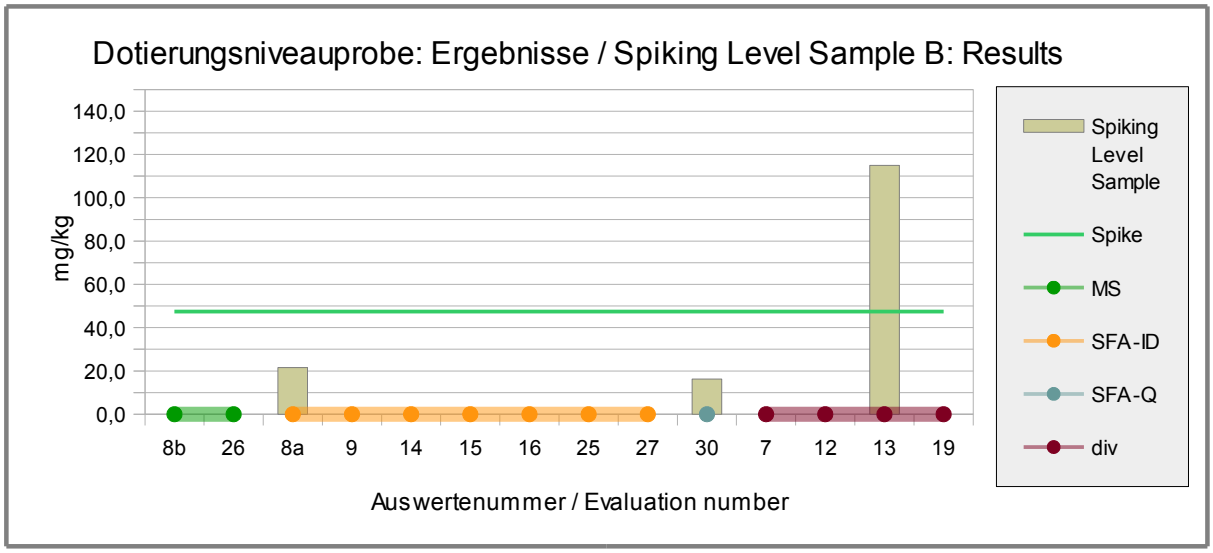

Abb./Fig. 20: PCR Results Sesame
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Sesame:
Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
8b					MS	
26					MS	
8a	21,5	45	58,5	32	SFA-ID	
9					SFA-ID	
14					SFA-ID	
15					SFA-ID	
16					SFA-ID	
25	>0.4		>0,4		SFA-ID	
27					SFA-ID	
30	16,2	34	4,0		SFA-Q	
7					div	
12					div	
13	115	243	145	**80**	div	
19			92	**51**	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**2**
Percent in RA	**0**	Percent in RA	**67**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

MS = Microsynth

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

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

div = not indicated / other method

Comments:

For the spiking level sample none of the participants obtained a recovery rate by PCR within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B two recovery rates were within the recommended range.

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Mustard

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
AQ	6	26.06.17	negative	<2	negative	<2	positive	126,8	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	18	13/7-24/7/17	negative	<1	negative	<1	positive	>60	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
AQ	28	26/06	negative	<2.0	negative	<2.0	positive	85,6	Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
BC	23	03.07.17	negative	<2	negative	<2	-	110,8	Mustard	BioCheck ELISA Mustard-Check
ES	25		negative	<1	negative	<1	positive	16	Mustard Protein	ELISA Systems Mustard ESMUS-48
IL	1	27.06.17	negative	<1	positive	2,02	positive	95,1	Mustard	Immunolab Mustard ELISA
NL	4		negative		positive	<0,5	positive	20,8	Mustard	Romer Labs NutriLinia NC-6008
RS-F	2	11.07.17	negative		negative		positive		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	3	16.08.17	negative	<0.5	negative	<0.5	positive	75,64	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	7	05.07.17	positive	2,21	negative	<0,5	positive	76,27	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	9		negative		negative		positive		Please select!	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	13		negative	-	positive	<0.5	positive	60	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	16	24.07.17	negative		positive		positive		Please select!	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	17	28.07.17	negative	<0,5	negative	<0,5	positive	>13,5	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	19	05.07.17	negative	<0.5 (<LOQ)	negative	<0.5 (<LOQ)	positive	78	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	22	28.07.17	negative	<0,5	negative	<0,5	positive	76	Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	27		negative	< 0,5	negative	< 0,5	positive	73,5	Mustard Powder	RIDASCREEN® FAST Mustard, R6152, Fa. R-Biopharm
VT	10	04.10.02	negative		negative		positive	112	Mustard	Veratox Mustard, Neogen
VT	11		negative	<2.5	negative	<2.5	-		Mustard	Veratox Mustard, Neogen
VT	21		negative		negative		-		Mustard Protein	Veratox Mustard, Neogen
VT	29	11.07.17	negative	<2.5	negative	<2.5	positive	73,3	Mustard	Veratox Mustard, Neogen

Continuation ELISA Mustard:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6				
AQ	18	Mustard	According to Manual	no	
AQ	28			No	
BC	23	Polyclonal	PBS, 15min, 60°C	No	
ES	25			no	
IL	1	polyclonal			
NL	4	Mustard	As Per Kit Instructions	yes	Ab against white mustard; cross-reactivity to black mustard : < 5%, to brown mustard : <5%
RS-F	2	Mustard protein		yes	
RS-F	3	Mustard	As Per Kit Instructions	Yes	Negative using ELISA, but Positive using PCR
RS-F	7	Mustard	As Per Kit Instructions	yes	
RS-F	9		As Per Kit Instructions	yes	
RS-F	13		As Per Kit Instructions	yes	below LOQ, also with AgraQuant Kit
RS-F	16	Mustard protein	As Per Kit Instructions	no	
RS-F	17			no	
RS-F	19			yes	
RS-F	22	Mustard protein	AEP /10 min/ 60^C	yes	
RS-F	27		As Per Kit Instructions	yes	
VT	10			no	Method under validation
VT	11			yes	
VT	21		5g in 125mL extraction sol'n for 15mins at 60°C 3 steps of 10mins incubation assay	yes	LOQ = 2.5ppm mustard seed protein
VT	29				

5.1.2 ELISA: Sesame

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
AQ	6	23.06.17	negative	<2	negative	<2	positive	39,8	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
AQ	18	13/7-24/7/17	negative	<0,2	positive	1,2	positive	60	Sesame	AgraQuant ELISA Sesame COKAL1948, RomerLabs
BC	23	03.07.17	negative	<2	negative	<2	-	34,6	Sesame	BioCheck ELISA Sesame-Check
BK	4		negative		positive	76,5	positive	330	Sesame	BioKits Sesame Protein Assay Kit, Neogen
BK	10	04.10.02	negative		positive	48,5	positive	198	Sesame	BioKits Sesame Protein Assay Kit, Neogen
ES	5		negative	<0.5	negative	<0.5	positive	1,64	Sesame seed protein	ELISA Systems Sesame ESSESRD-48
ES	9		negative		negative		positive		Please select!	ELISA Systems Sesame ESSESRD-48
ES	11		negative	<0.5	negative	<0.5	-		Sesame protein	ELISA Systems Sesame ESSESRD-48
ES	17	28.07.17	negative	<0,5	negative	<0,5	positive	1,3	Sesame protein	ELISA Systems Sesame ESSESRD-48
ES	21		negative		negative		-		Sesame protein	ELISA Systems Sesame ESSESRD-48
ES	25		negative	<0.5	negative	<0.5	positive	1,9	Sesame protein	ELISA Systems Sesame ESSESRD-48
ES	28	19/07	negative	<0.5	negative	<0.5	positive	0,75	Sesame protein	ELISA Systems Sesame ESSESRD-48
ES	29	11.07.17	negative	<0.5	negative	<0.5	positive	1,1	Sesame protein	ELISA Systems Sesame ESSESRD-48
IL	1	27.06.17	negative	<0.2	positive	3,78	positive	50,6	Sesame	Immunolab Sesame ELISA
RS-F	2	11.07.17	negative		positive	10,3	positive		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	3	16.08.17	negative	<2.5	positive	19,06	positive	209,9	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	7	15.08.17	negative	<2,5	positive	7,76	positive	197,06	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	13		negative	-	positive	10	positive	130	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	19	04.07.17	-		positive	10	positive	140	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	22	07.07.17	negative	<2,5	positive	8,5	positive	140	Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
VT	11		negative	<2,5	positive	98	-		Sesame	Veratox Sesame Allergen, Neogen
VT	24	07.07.17	negative	<6.25	positive	38	positive	349	Sesame	Veratox Sesame Allergen, Neogen

Continuation ELISA Sesame:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6				
AQ	18	Sesame	According to Manual	no	
BC	23	Polyclonal	PBS, 15min, 60°C	No	
BK	4	Sesame	As Per Kit Instructions	yes	
BK	10			no	Method under validation
ES	5				
ES	9		As Per Kit Instructions	yes	
ES	11			yes	
ES	17			no	
ES	21		1g in 50mL extraction sol'n for 15 mins at 60°C 4 steps of 15mins incubation assay	yes	LOQ =0.5ppm sesame seed protein
ES	25			no	
ES	28			Yes	
ES	29				
IL	1	polyclonal			
RS-F	2	Sesame protein		yes	
RS-F	3	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	7	Sesame	As Per Kit Instructions	yes	Fast Sesame w ith new calibration material LOT-Nr. 15287
RS-F	13		As Per Kit Instructions	yes	
RS-F	19	Ses i 1-7		yes	
RS-F	22	Sesame protein	AEP /10 min /60°C	yes	
VT	11			no	
VT	24		Manufacturer instruction	yes	

5.1.3 PCR: Celery

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
ASU	7	12.07.17	negative		positive		positive		Celery-DNA	ASU §64 Methode/method
ASU	12	29.06.17/ 17.07.17	negative		positive		positive		Please select!	ASU §64 Methode/method
ASU	16	13.07.17	negative		positive		positive		Celery-DNA	ASU §64 Methode/method
MS	8b		negative		positive		positive		Please select!	Microsynth
MS	26		negative		positive		-		Please select!	Microsynth
SFA-4p	30a	27.06.17	negative	-	positive	-	positive	-	Please select!	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	30.06.17	negative	<1	positive	121,32	positive	118,77	Celery	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	8a		negative		positive	61	positive	24	Celery	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	9		negative		positive		positive		Please select!	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	14	14.08.17	negative		positive		positive		Please select!	Sure Food Allergen ID, Cellery, R-Biopharm / Congen
SFA-ID	15		negative		positive		positive		Celery-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	20		negative		positive		-		Please select!	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	25	xx	negative	<0.4	positive	>0.4	positive	>0.4	Celery-DNA	other: Please select
SFA-ID	27a		negative		positive		positive		Celery	SureFood® ALLERGEN ID Celery Fa. Congen
SFA-Q	18	24.07.17	negative	<0,4	positive	20,28	positive	12,85	Celery-DNA	Sure Food Allergen Quant, R-Biopharm / Congen
SFA-Q	27b		negative	< 1,0	positive	5,78	positive	14,5	Celery	SureFood® ALLERGEN QUANT Celery, Fa. Congen
SFA-Q	30b	27.06.17	negative	-	positive	5	positive	13,2	Celery seed, dried.	Sure Food Allergen Quant, R-Biopharm / Congen
div	6	16.08.17	negative		negative		positive		Celery-DNA	in house method
div	13		negative	-	positive	55	positive	20	Celery seed, dried	Lauber et al. 2015
div	19	28.06.17	negative	n.n.	positive	130	positive		Celery	in house method

Continuation PCR Celery:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ASU	7	Mannitoldehydrogenase (101 bp)	CTAB based extraction with clean-up by Wizard-Kit (Promega); Real-time PCR: 45 Cycles	yes	
ASU	12	Celery-DNA	Extraction by Wizard Resin/ Real Time PCR		
ASU	16	Mannitol-Dehydrogenase	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes (DIN EN ISO/IEC 17025:2005)	
MS	8b	Celery	CTAB/QIAquick/Real-Time PCR	yes	
MS	26	Celery	Macherey Nagel Nucleo Spin Food with optimizations: increased sample weight, buffer changing (Washing with Lysis Buffer) RNase-Step, Chloroform-Step, 2xCQW; RealTime PCR with 45 Cycles, Decontamination step with UNG; individual Thermoprofile; Inhibition control	yes	Problems detecting Celery-DNA in Spiking Level Sample - probably matrix-effects; strong inhibition
SFA-4p	30a	Celery	S3401 SureFood®ALLERGEN 4plex Soya/Celery/Mustard+IAC, LOD 0,4 mg/kg, Extraction with S1053 SureFood® PREP Advanced, Protocol 1	yes	-
SFA-ID	3	As Per Kit Instructions	As Per Kit Instructions	Yes	Quantified using Quantard 40 Standard
SFA-ID	8a	Celery	CTAB/QIAquick/Real-Time PCR	yes	
SFA-ID	9		As Per Kit Instructions	yes	Sample B: Traces
SFA-ID	14			yes	LOD 0,4 ppm
SFA-ID	15			yes	
SFA-ID	20			yes	operator: Poletti
SFA-ID	25	celery Art.-No. S3105 Congen		no	
SFA-ID	27a		SureFood® PREP Advanced, Congen, Protocol 2	yes	
SFA-Q	18	Celery	Real time PCR	no	
SFA-Q	27b		SureFood® PREP Advanced, Congen, Protocol 1	no	
SFA-Q	30b	Celery	S3205 SureFood® ALLERGEN QUANT Celery, LOD 0,4 mg/kg, LOQ 1 mg/kg; Extraction with S1053 SureFood® PREP Advanced, Protocol 1	nein	-
div	6		gel electrophoresis, LOD 10ppm		
div	13	Mannitoldehydrogenase Gene	ReliaPrep™ , Promega	yes	
div	19	5'-(FAM)-aac aga taa cgc tga ctc atc aca ccg-(TAMRA)-3'	Wizard/Real Time PCR/45 Cycles	yes	

5.1.4 PCR: Mustard

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
MS	8b		negative		positive		positive		Please select!	Microsynth
SFA-4p	30a	27.06.17	negative	-	positive	-	positive	-	Please select!	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	30.06.17	negative	<1	positive	82,94	positive	73,65	Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	8a		negative		positive	204	positive	60	Mustard	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	9		negative		positive		positive		Please select!	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	14	14.08.17	negative		positive		positive		Please select!	Sure Food Allergen ID, Mustard, R-Biopharm / Congen
SFA-ID	15		negative		positive		positive		Mustard-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	25		negative	<0.4	positive	>0.4	positive	>0.4	Mustard-DNA	other: Please select!
SFA-ID	27		negative		positive		positive		Mustard	SureFood® ALLERGEN ID Mustard Fa. Congen
div	12	29.06.17	negative		positive		positive		Please select!	Real-Time PCR
div	13		negative	-	positive	65	positive	45	Mustard	Lauber et al. 2015
div	16	28.06.17	negative		positive		positive		Mustard-DNA	Mustorp et al. 2008 Eur Food Res Technol. 226: 771-778
div	19	28.06.17	negative	n.d.	positive	230	positive		Mustard	in house method
div	26		negative		positive		positive		Please select!	in house method
div	30b	27.06.17	negative	-	positive	3,9	positive	13,1	Mustard	in house method

Continuation PCR Mustard:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
MS	8b	Mustard	CTAB/QIAquick/Real-Time PCR	yes	
SFA-4p	30a	Mustard	S3401 SureFood®ALLERGEN 4plex Soya/Celery/Mustard+IAC, LOD 0,4 mg/kg, Extraction with S1053 SureFood® PREP Advanced, Protocol 1	yes	-
SFA-ID	3	As Per Kit Instructions	As Per Kit Instructions	Yes	Quantified using Quantard 40 Standard
SFA-ID	8a	Mustard	CTAB/QIAquick/Real-Time PCR	yes	
SFA-ID	9		As Per Kit Instructions	yes	Sample B: Traces
SFA-ID	14			yes	LOD 0,4 ppm
SFA-ID	15			no	
SFA-ID	25	SureFood® ALLERGEN Mustard Art.-No. S3109 Congen		no	
SFA-ID	27		SureFood® PREP Advanced, Congen, Protocol 2	yes	
div	12	Mustard -DNA	Extraktion mit Wizard Resin/ Real Time PCR		
div	13	S. alba, B. nigra, B. juncea	ReliaPrep™ , Promega	yes	only white Mustard (S. alba) pos.
div	16	Major allergen sin a1	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes (DIN EN ISO/IEC 17025:2005)	
div	19	5'-(FAM)-TTM GAC AAC AGY TGG GGC AGC AGG G-(BHQ-1)-3'	Wizard/Real Time PCR/50 Cycles	yes	SinA1-Gene from Sinapis alba
div	26	Mustard	Macherey Nagel Nucleo Spin Food with optimizations: increased sample weight, buffer changing (Washing with Lysis Buffer) RNase-Step, Chloroform-Step, 2xCQW; RealTime PCR with 45 Cycles, Decontamination step with UNG; individual Thermoprofile; Inhibition control	yes	Sinapis alba detectable; Brassica juncea/nigra not detected
div	30b	Mustard	quantitative in house method, LOD 0,4 mg/kg, LOQ 2,5 mg/kg; Extraction with S1053 SureFood® PREP Advanced, Protocol 1	no	-

5.1.5 PCR: Sesame

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Manufacturer
MS	8b		negative		positive		positive		Please select!	Microsynth
MS	26		negative		positive		positive		Please select!	Microsynth
SFA-ID	8a		negative		positive	58,5	positive	21,5	Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	9		negative		positive		positive		Please select!	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	14	14.08.17	negative		positive		positive		Please select!	Sure Food Allergen ID, Sesam, R-Biopharm / Congen
SFA-ID	15		negative		positive		positive		Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	16	28.06.17	negative		positive		positive		Sesame-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	25		negative	<0.4	positive	>0.4	positive	>0.4		other: Please select!
SFA-ID	27		negative		positive		positive		Sesame	SureFood® ALLERGEN ID Sesam, Fa. Congen
SFA-Q	30	27.06.17	negative	-	positive	4	positive	16,2	Sesame	Sure Food Allergen Quant, R-Biopharm / Congen
div	7	12.07.17	negative		positive		positive		Sesame-DNA	Mustorp et al., 2008 Eur Food Res Technol
div	12	29.06.17	negative		positive		positive		Please select!	Mustorp et al.2007
div	13		negative	-	positive	145	positive	115	Sesame	Lauber et al. 2015
div	19	28.06.17	negative	n.d.	positive	92	positive		Sesame	in house method

Continuation PCR Sesame:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
MS	8b	Sesame	CTAB/QIAquick/Real-Time PCR	yes	
MS	26	Sesame	Macherey Nagel Nucleo Spin Food with optimizations: increased sample weight, buffer changing (Washing with Lysis Buffer) RNase-Step, Chloroform-Step, 2xCQW; RealTime PCR with 45 Cycles, Decontamination step with UNG; individual Thermoprofile; Inhibition control	yes	
SFA-ID	8a	Sesam	CTAB/QIAquick/Real-Time PCR	yes	
SFA-ID	9		As Per Kit Instructions	yes	Sample B: Traces
SFA-ID	14			yes	LOD 0,4 ppm
SFA-ID	15			no	
SFA-ID	16	Sesame	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	yes (DIN EN ISO/IEC 17025:2005)	
SFA-ID	25	SureFood® ALLERGEN Sesame Art.-No. S3108 Congen		no	Sesame - DNA -
SFA-ID	27		SureFood® PREP Advanced, Congen, Protocol 2	yes	
SFA-Q	30	Sesame	S3208 SureFood® ALLERGEN QUANT Sesame, LOD 0,4 mg/kg, LOQ 1 mg/kg; Extraction by S1053 SureFood® PREP Advanced, Protocol 1	yes (qualitative)	-
div	7	2S-albumin (64 bp)	CTAB based extraction with clean-up by Wizard-Kit (Promega); Real-time PCR: 45 Cycles	yes	
div	12	Sesame -DNA	Extraction by Wizard Resin/ Real Time PCR		
div	13	Oleosin mRNA	ReliaPrep™ , Promega	yes	
div	19	5'-(FAM)-cat ctt ggt ccc cgc cgc cct aat-(BHQ-1)-3'	Wizard/Real Time PCR/50 Cycles	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 04-2017 Spiking Level Sample

Weight whole sample	1,09	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	35,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	101	39,9
2	5,00	98	39,2
3	5,03	80	31,8
4	5,11	80	31,3
5	5,10	80	31,4
6	5,09	85	33,4
7	5,08	92	36,2
8	4,97	91	36,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	88,4	Particles
Standard deviation	8,83	Particles
χ^2 (CHI-Quadrat)	6,18	
Probability	**52**	%
Recovery rate	99	%

Normal distribution		
Number of samples	8	
Mean	35,0	mg/kg
Standard deviation	3,49	mg/kg
rel. Standard deviaton	10,0	%
Horwitz standard deviation	9,4	%
HorRat-value	**1,1**	
Recovery rate	99	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 04-2017***
PT name	***Allergens IV: Celery, Mustard and Sesame in Sausage***
Sample matrix (processing)	***Samples A + B:*** *Sausage (heated)/ ingredients: beef, pork meat, water, potato powder, salt, sodium citrate, 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: cooled 2 - 10°C (long term < -18°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *celery, mustard, sesame* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. From* ***Samples A + B*** *the* ***total sample amount*** *should be* ***homogenized****.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest August 18th 2017***
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, PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
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