Proficiency Tests

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Evaluation Report
proficiency test

DLA 04/2016

Allergens IV:

Celery, Mustard and Sesame

in Asparagus Soup Powder

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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.

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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 for the detection of allergens in the range of mg/kg and one spiking material sample were provided for analysis. The spiking material sample contains the respective allergenic ingredients in the range of 1-10 % and was added to the spiked PT-sample. The results of the spiking material 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 is a common in commerce "asparagus cream soup" instant powder. The basic composition of both sample A and sample B was the same (see table 1). After crushing and homogenization of the basic mixture the spiked sample A was prepared as follows:
The spiking material containing the allergenic ingredients celery, mustard and sesame was added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 4 additional steps and mechanically homogenized in each case until the total quantity had been reached.

The composition of the spiking material sample and the amounts of allergens in sample A is given in table 2. The allergenic raw materials were crushed and sieved (mesh 400 µm) or sieved by means of a centrifugal mill (mesh 500 µm) prior to use.

After homogenization the samples were portioned to approximately 25 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Asparagus Cream Soup, Powder Ingredients: Palm fat, maltodextrin, modified starch, Wheat flour, salt, sugar, milk sugar, flavorings, Yeast extract, milk protein, sunflower oil, Asparagus (0.5%), onions, acidifying agent: citric acid, curcuma Nutrients per 100 g: Protein 3,8 g, Carbohydrates 60 g, Fat 24 g	99,4 g/100 g	100 g/100 g
Spiking Material Sample	0,56 g/100 g	-

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Amounts in Sample A**
Potato flour Nutrients per 100g: Protein 0 g	93 %	0,56 %
Celery seed: - as Celery powder* - *thereof 20% total protein***	 9050 mg/kg (0,91 %) 1810 mg/kg	 51 mg/kg 10 mg/kg
Mustard, yellow (Sinapis alba): - as Mustard powder* - *thereof 30% total protein***	 9000 mg/kg (0,90 %) 2700 mg/kg	 50 mg/kg 15 mg/kg
Sesame, white: - as Sesame paste* - *thereof 23% total protein***	 13100 mg/kg (1,31 %) 2960 mg/kg	 78 mg/kg 18 mg/kg
additional ingredients: *Cashew paste, shrimps, dried, maltodextrin, sodium chloride, sodium sulfate and silicon dioxide*	< 5,00 %	< 0,03 %

*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)

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 10-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples showed a probability of 69% for the spiked sample A and of 98% for the spiking material sample. 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 Hor-Rat value of 1,1 and 0,6 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 fulfilled for sample A by all ELISA tests for mustard (Immunolab and Veratox) and sesame (Immunolab), respectively (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. 3.8 and 3.11) [3].

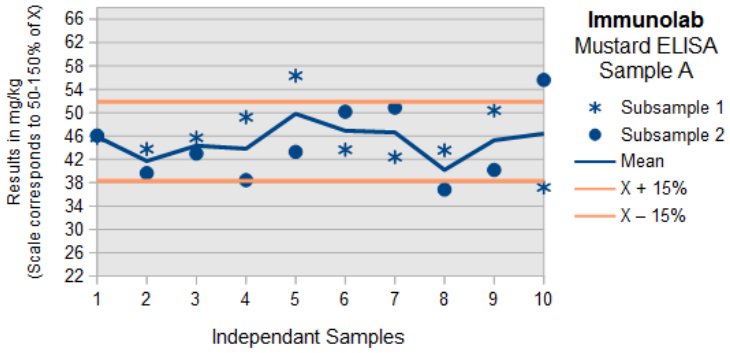
ELISA-Tests: Homogenität Senf / Homogeneity Mustard

Immunolab Mustard ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Mustard 45,1 ± 6,5 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	45,70	46,08	45,89
2	43,77	39,64	41,71
3	45,70	42,99	44,35
4	49,22	38,44	43,83
5	56,34	43,28	49,81
6	43,65	50,16	46,91
7	42,39	50,85	46,62
8	43,56	36,83	40,19
9	50,37	40,18	45,28
10	37,20	55,60	46,40

General average X	45,10	
SD of sample means Sx	2,75	6,1%
SD w ithin-samples Sw	6,76	15,0%
SD betw een-samples Ss	6,47	14,3%

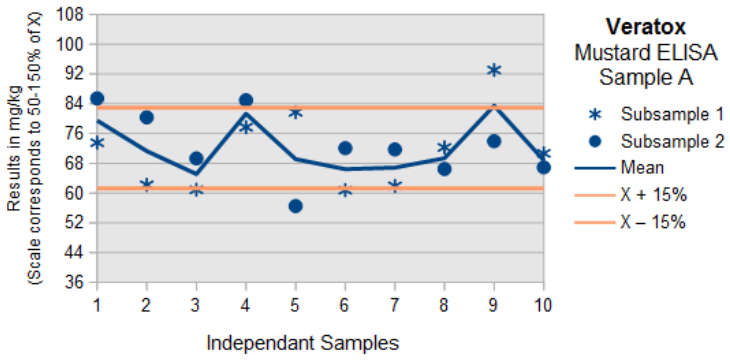


Neogen Veratox ELISA Mustard

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Mustard 72,1 ± 8,4 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	73,54	85,45	79,49
2	62,21	80,34	71,28
3	60,90	69,31	65,10
4	77,69	84,95	81,32
5	81,70	56,51	69,11
6	60,77	72,06	66,41
7	61,91	71,71	66,81
8	72,27	66,47	69,37
9	93,07	73,94	83,50
10	70,67	66,90	68,78

Allgemeiner Mittelw ert X	72,12	
Std.abw . d. Probenmittelw erte Sx	6,73	9,3%
Std.abw . innerhalb d. Proben Sw	9,66	13,4%
Std.abw . zw ischen d. Proben Ss	8,41	11,7%



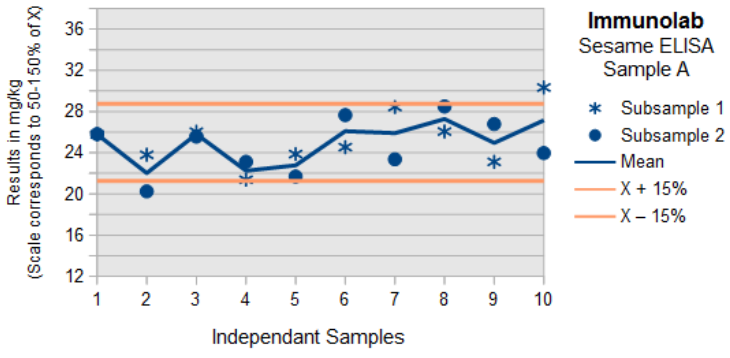
ELISA-Tests: Homogenität Sesam / Homogeneity Sesame

Immunolab Sesame ELISA

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

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	25,77	25,81	25,79
2	23,80	20,25	22,02
3	26,04	25,56	25,80
4	21,38	23,11	22,25
5	23,87	21,69	22,78
6	24,54	27,65	26,09
7	28,43	23,36	25,90
8	26,06	28,49	27,27
9	23,12	26,78	24,95
10	30,32	23,96	27,14

General average X	25,00	
SD of sample means Sx	1,96	7,8%
SD w ithin-samples Sw	2,41	9,6%
SD betw een-samples Ss	1,97	7,9%



2.2 Sample shipment and information to the test

The portions of test material (sample A and sample B as well as the spiking material sample) were sent to every participating laboratory in the 32nd week of 2016. The testing method was optional. The tests should be finished at October 7th 2016 the latest.

With the cover letter along with the sample shipment the following information was given to participants:
There are two different test samples Sample A and Sample B ***Soup Powder.*** *Both are possibly containing the allergenic foods celery, mustard and/or sesame in the range of mg/kg.*
Additionally a „Spiking Material Sample" is provided which was used for the spiking of the positive samples (A or B). It contains 1-10% of the allergenic items in potato flour and should be analysed like a normal sample (eventually diluted).
The homogeneity of the material was tested. Every suitable method for detection or determination of the analytes may be applied.
In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

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 specifity, 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.
One participant submitted no results. All other 27 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.

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-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 3a: 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} [27-28]

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 11 – 33% for the ELISA methods and 15 – 43% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 3a and 3b).

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 3b: 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} [29-33]

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 3: 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 4: 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 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 3.6.1.

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 material 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 2. 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

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 analyte are reported for sample A and afterwards for sample B. The results of the spiking material sample are reported together with the referring spiked sample in the recovery section.

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})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ i}}$
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 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		
25	positive		positive		1/1 (100%)	4L	
6	positive		positive		1/1 (100%)	ASU	
11	positive	-	negative	-	1/1 (100%)	ASU	
20	positive		negative		1/1 (100%)	ASU	
22	positive	30	positive	< 20	1/1 (100%)	ASU	
23	positive	--	positive	--	1/1 (100%)	ASU	
26	positive		negative		1/1 (100%)	ASU	
14	positive	10,18	positive	3,42	1/1 (100%)	FP	
9	positive		negative		1/1 (100%)	MS	
15b	positive	9,5	positive	15,5	1/1 (100%)	MS	
15a	positive	16	positive	4,5	1/1 (100%)	SFA-4p	
17	positive	99,06	positive	23,32	1/1 (100%)	SFA-ID	
18	positive		positive		1/1 (100%)	SFA-ID	
19	positive	>0,4	positive	>0,4	1/1 (100%)	SFA-ID	
10	positive	14,75	positive	7,5	1/1 (100%)	SFA-Q	
21	positive	9,9	positive	1,1	1/1 (100%)	SFA-Q	
1	negative		positive		0/1 (0%)	div	
3	positive		negative		1/1 (100%)	div	
5	negative	<1	negative	<1	0/1 (0%)	div	
8	positive	150	positive	140	1/1 (100%)	div	

	Sample A		Sample B	
Number positive	18		14	
Number negative	2		6	
Percent positive	90		70	
Percent negative	10		30	
Consensus value	positive		none	

Methods:

4L = 4LAB Diagnostics
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
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 value for sample A is in agreement with the spiking of sample A. For sample B no consensus value of 75% agreement was obtained. 14 participants detected celery in the non-spiked sample B by PCR methods.

Quantitative valuation of results: Sample A

Evaluation number	Celery	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
25			4L	
6			ASU	
11	-		ASU	
20			ASU	
22	30	4,8	ASU	
23	--		ASU	
26			ASU	
14	10,18	-1,0	FP	
9			MS	
15b	9,5	-1,2	MS	
15a	16	0,7	SFA-4p	
17	99,06		SFA-ID	Result excluded
18			SFA-ID	
19	>0,4		SFA-ID	
10	14,75	0,3	SFA-Q	
21	9,9	-1,1	SFA-Q	
1			div	
3			div	
5	<1		div	
8	150		div	Result excluded

Methods:
4L = 4LAB Diagnostics
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
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

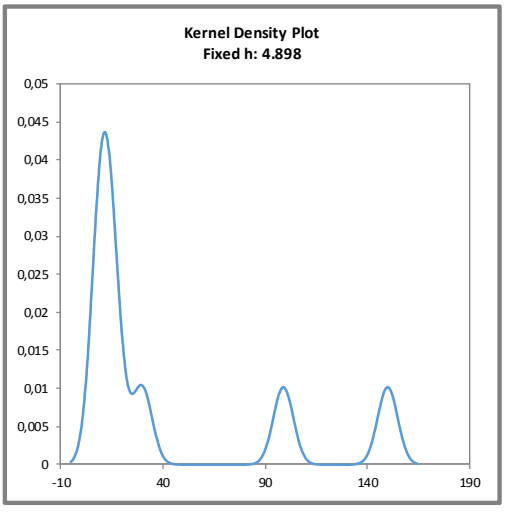


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a normal distribution for 5 results besides an additional shoulder and two single results (outliers).

Characteristics: Quantitative evaluation Celery

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	15,1
Median	12,5
Robust Mean (X)	**13,7**
Robust standard deviation (S*)	**5,51**
Target range:	
Target standard deviation σ_{pt}	**3,43**
lower limit of target range	**6,85**
upper limit of target range	**20,6**
Quotient S/σ_{pt}*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*2,81*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,82*
Results in the target range	*5*
Percent in the target range	*83*

Comments to the statistical characteristics and assigned values:

After elimination of two outliers the evaluation of all methods showed a normal variability of results. The quotient S/σ_{pt} was below 2,0.*
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 formally given. This conclusion is limited, because there are only a few results at all and for each method. Therefore the evaluation of results by z-scores has only limited significance.

The robust mean of the evaluation was clearly below the spiking level of celery to sample A. It should also taken into account that sample B (the basic matrix of sample A) also contained celery (s. "Recovery rates of Celery" p.25).

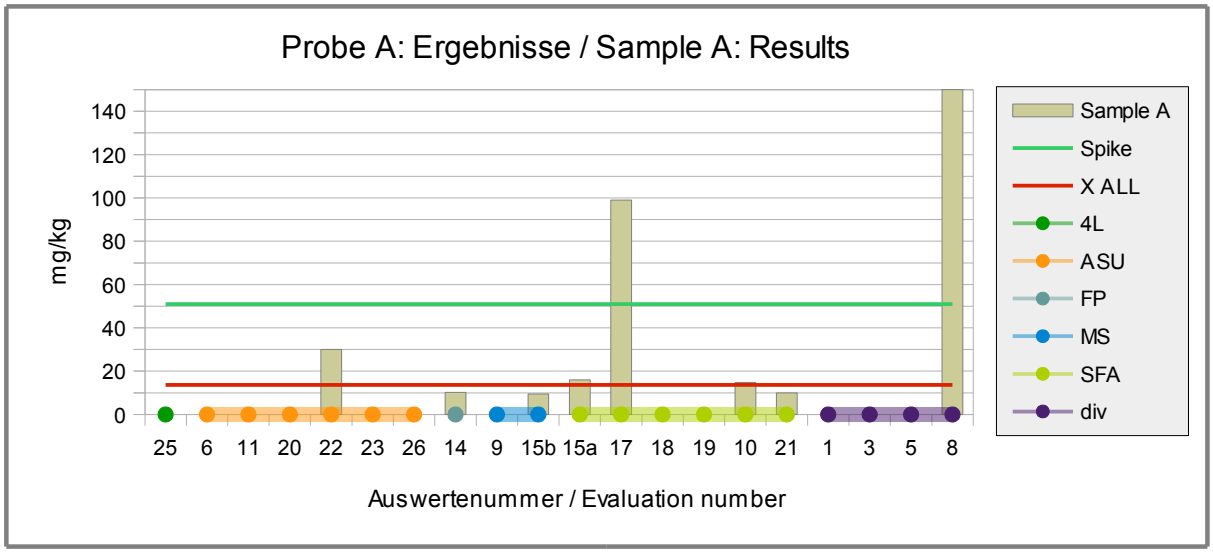


Abb./Fig. 2: PCR Results Celery
green line = Spiking level
red line = Assigned value robust mean all included results
round symbols = Applied methods (see legend)

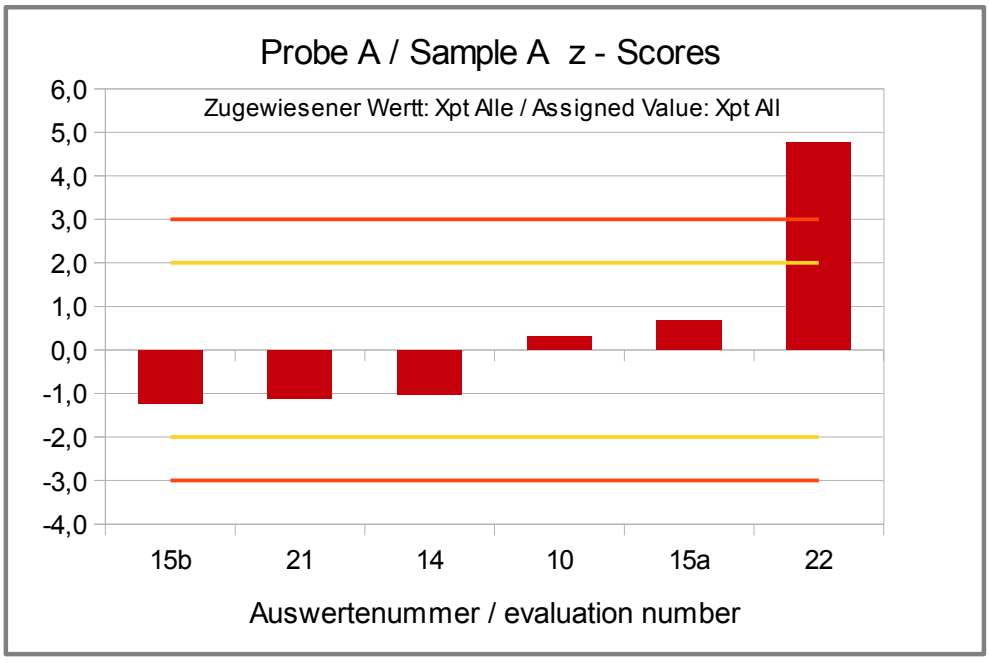


Abb./Fig. 3:
z-Scores (PCR Results Celery)
Assigned value robust mean of all results

Recovery Rates for Celery:
Spiking Material Sample and Sample A (- Probe B)

Evaluation number	Spiking material	Recovery rate	Sample A minus B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
25					4L	
6					ASU	
11	-		-		ASU	
20					ASU	
22	8400	**93**	30	**59**	ASU	
23	--		--		ASU	
26					ASU	
14			6,76	13	FP	
9					MS	
15b	8676	**96**	-6	-12	MS	Single results Spike: 8948/8403 °
15a	6499	**72**	11,5	23	SFA-4p	Single results Spike: 6860/6137 °
17	19397	214	75,74	**149**	SFA-ID	
18					SFA-ID	
19	>0,4		>0,4		SFA-ID	
10	5223	**58**	7,25	14	SFA-Q	
21	7597	**84**	8,8	17	SFA-Q	
1					div	
3					div	
5	>1		<1		div	
8	104200	1151	10	20	div	

° Mean calculated by DLA

RA*	50-150 %	RA*	50-150 %
Number in RA	**5**	Number in RA	**2**
Percent in RA	**71**	Percent in RA	**25**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
4L = 4LAB Diagnostics
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
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 75% (5) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For valuation of the food matrix sample A produced with the spiking material sample the content in the matrix (corresponds to results for sample B) were deducted. Afterwards 2 of 8 recovery rates were in the range of acceptance.

Quantitative valuation of results: Sample B

Evaluation number	Celery	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
25			4L	
6			ASU	
11	-		ASU	
20			ASU	
22	< 20		ASU	
23	--		ASU	
26			ASU	
14	3,42		FP	
9			MS	
15b	15,5		MS	
15a	4,5		SFA-4p	
17	23,32		SFA-ID	
18			SFA-ID	
19	>0,4		SFA-ID	
10	7,5		SFA-Q	
21	1,1		SFA-Q	
1			div	
3			div	
5	<1		div	
8	140		div	Result excluded

Methodes:

4L = 4LAB Diagnostics
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
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:

A kernel density estimation was not done due to the number of < 8 results.

Characteristics: Quantitative evaluation Celery

Sample B

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	9,22
Median	6,00
Robust Mean (X)	**9,22**
Robust standard deviation (S*)	**9,66**
Target range:	
Target standard deviation σ_{pt}	
lower limit of target range	
upper limit of target range	
Quotient S*/σ_{pt}	
Standard uncertainty $U_{(Xpt)}$	
Quotient $U_{(Xpt)}/\sigma_{pt}$	
Results in the target range	
Percent in the target range	

Comments to the statistical characteristics and assigned values:

Even after elimination of an outlier the evaluation of all methods showed an increased variability of results. The quotient S*/σ_{pt} *was higher than 4. Therefore no valuation of results by target range and z-scores was performed.*

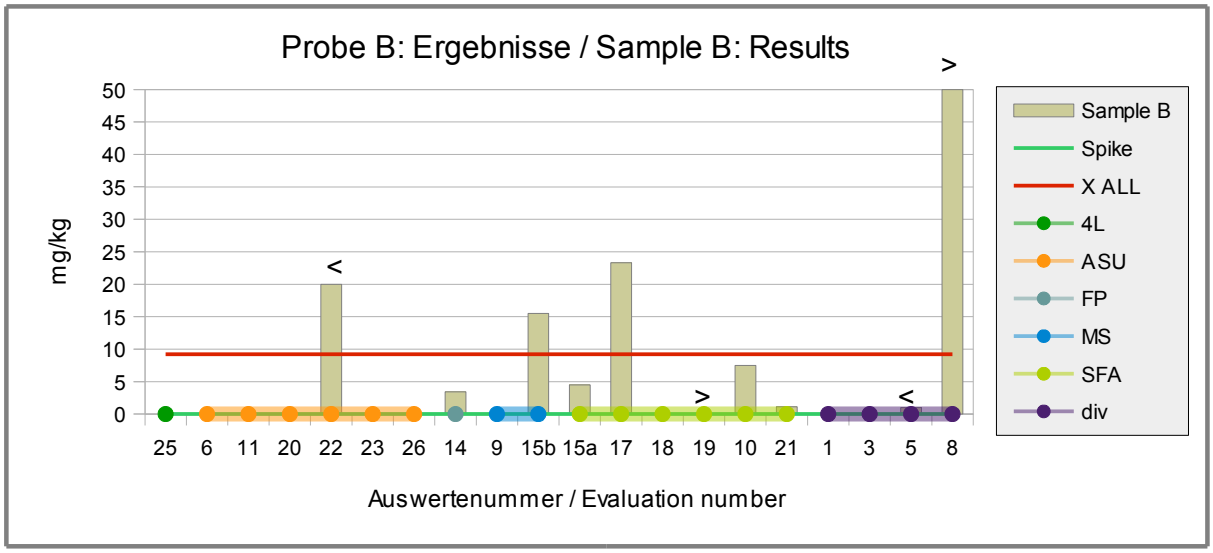


Abb./Fig. 4: PCR Results Celery
red line = Assigned value robust mean all included results
round symbols = Applied methods (see legend)

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		
1	positive	40,4	negative	< 2	2/2 (100%)	AQ	
24	positive	48,55	negative	< 2	2/2 (100%)	AQ	
7	positive	31,1	negative	< 2	2/2 (100%)	BC	
14	positive	65,5	negative	< 1	2/2 (100%)	ES	result converted *
19	positive	49	negative	< 1	2/2 (100%)	ES	result converted *
25	positive	72	negative		2/2 (100%)	ES	result converted *
27	positive	52	negative	< 1	2/2 (100%)	IL	
6	positive	8,8	negative	< 2,50	2/2 (100%)	NL	
5	positive	60,6	positive	0,88	1/2 (50%)	RS	
10	positive	51	positive	1,79	1/2 (50%)	RS	
16	positive	52,83	positive	0,83	1/2 (50%)	RS	
17	positive	75,85	negative	< 0.5	2/2 (100%)	RS	
18	positive	76,9	positive	0,91	1/2 (50%)	RS	
2	positive	68	negative	nd	2/2 (100%)	VT	
3	positive	50	negative	< 2.5	2/2 (100%)	VT	
4	positive	96	negative	< 2,5	2/2 (100%)	VT	
11	positive	133	negative	< 2,5	2/2 (100%)	VT	
12	positive	102	negative	nd	2/2 (100%)	VT	
13	positive	54	negative	< 2.5	2/2 (100%)	VT	

* calculation see p. 18

	Sample A		Sample B	
Number positive	19		4	
Number negative	0		15	
Percent positive	100		21	
Percent negative	0		79	
Consensus value	positive		negative	

Methods:

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

Comments:
The consensus values are in agreement with the spiking of sample A. All results for sample B were < 2,5 mg/kg. There were 4 positive results by method RS in the range of the limit of quantification.

Quantitative valuation of results: Sample A

Evaluation number	Mustard	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
1	40,4	-1,4			AQ	
24	48,55	-0,8			AQ	
7	31,1	-2,0			BC	
14	65,5	0,3			ES	result converted *
19	49	-0,8			ES	result converted *
25	72	0,7			ES	result converted *
27	52	-0,6			IL	
6	8,8	-3,4			NL	
5	60,6	0,0	-0,2		RS	
10	51	-0,7	-0,8		RS	
16	52,83	-0,5	-0,7		RS	
17	75,85	1,0	0,8		RS	
18	76,9	1,0	0,8		RS	
2	68	0,5		-0,8	VT	
3	50	-0,7		-1,6	VT	
4	96	2,3		0,6	VT	
11	133	4,7		2,3	VT	
12	102	2,7		0,9	VT	
13	54	-0,5		-1,4	VT	

* calculation see p. 18

Methods:

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

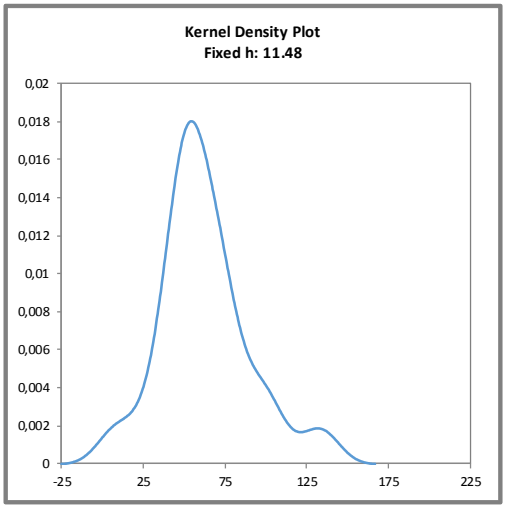


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 a shoulder at < 25 mg/kg (method NL) and two shoulders at > 90 mg/kg (method VT).

Characteristics: Quantitative evaluation Mustard

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$	$X_{pt_{METHOD\ VT}}$
Number of results	19	5	6
Number of outliers	1	0	0
Mean	62,5	63,4	83,8
Median	54,0	60,6	82,0
Robust Mean (X)	**61,1**	**63,4**	**83,8**
Robust standard deviation (S*)	**23,1**	**14,0**	**36,5**
Target range:			
Target standard deviation σ_{pt}	**15,3**	**15,9**	**21,0**
lower limit of target range	**30,6**	**31,7**	**41,9**
upper limit of target range	**91,7**	**95,2**	**126**
Quotient S/σ_{pt}*	*1,5*	*0,88*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*6,63*	*7,83*	*18,6*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,43*	*0,49*	*0,89*
Results in the target range	*15*	*5*	*5*
Percent in the target range	*79*	*100*	*83*

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

Comments to the statistical characteristics and assigned values:

The evaluation of all methods and the evaluation of results from method RS and VT showed a normal to low variability of results, respectively. The quotients S/σ_{pt} were below 2,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were 7 different methods with in part only a few results.*

The robust means of the evaluation of all results and method RS were 122% and 127% of the spiking level of mustard to sample A and within the recommendations for the applied methods, while the robust mean of method VT was 168% and slightly above the recommendations (s. 3.4.3 and "Recovery rates of Mustard" p.34).

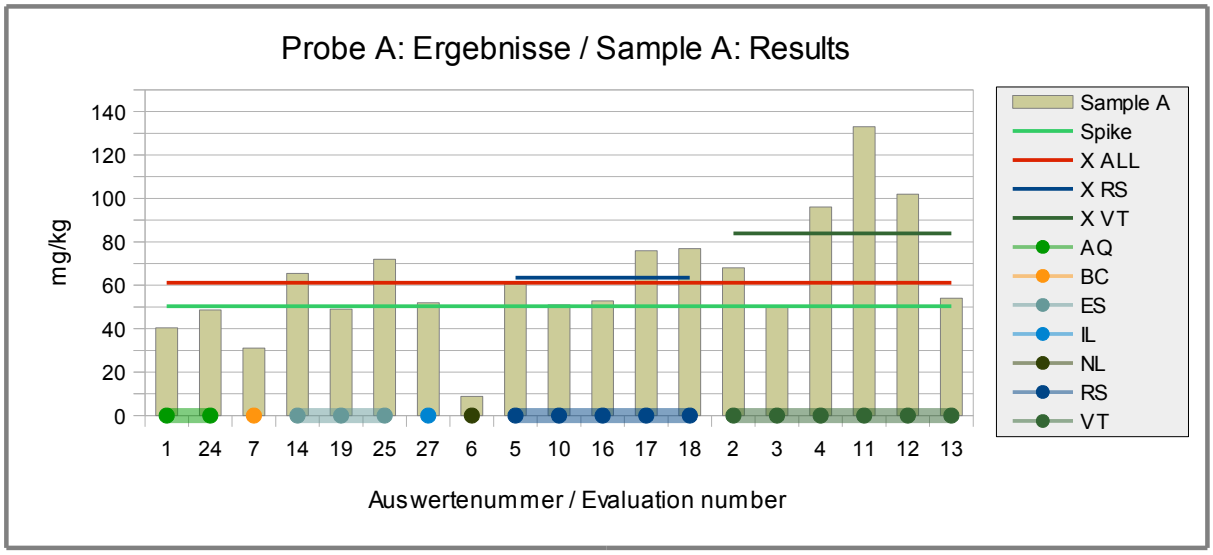


Abb./Fig. 6: ELISA Results Mustard
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS
dark green line = Assigned value robust mean results method VT
round symbols = Applied methods (see legend)

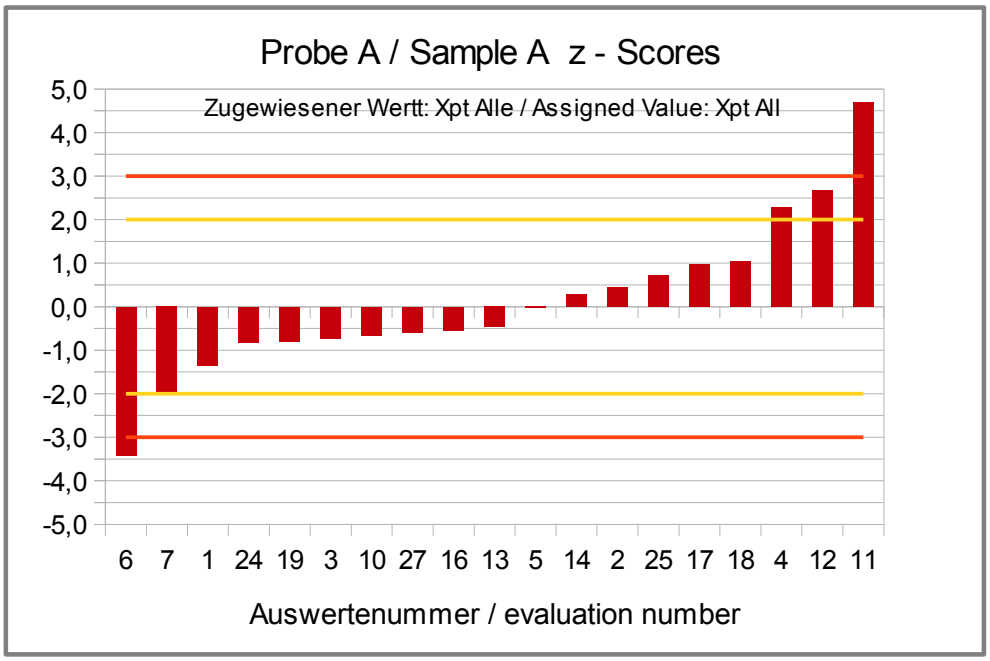


Abb./Fig. 7:
z-Scores (ELISA Results Mustard) Assigned value robust mean of all results

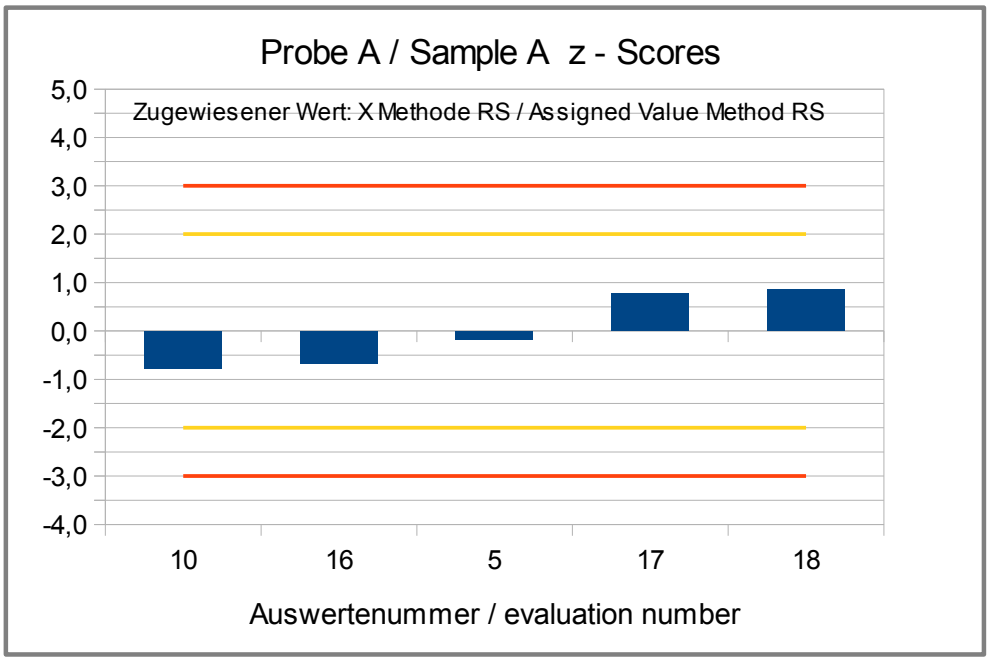


Abb./Fig. 8:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method RS (R-Biopharm, Ridascreen)

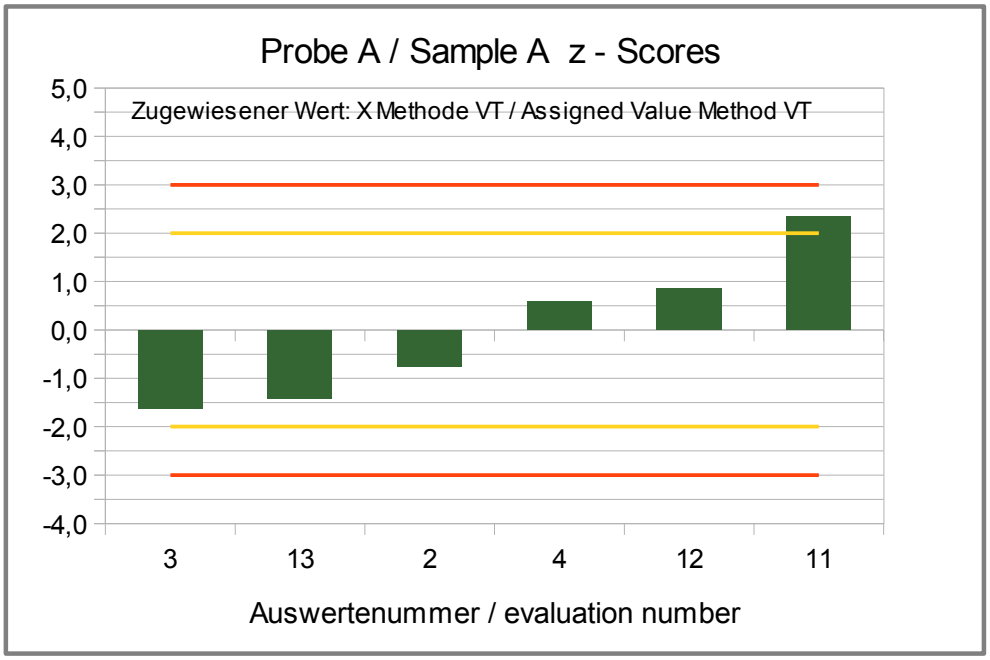


Abb./Fig. 9:
z-Scores (ELISA Results Mustard)
Assigned value robust mean of method VT (Veratox, Neogen)

Recovery Rates for Mustard:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	26081	290	40,4	**80**	AQ	
24	5946,05	**66**	48,55	**96**	AQ	
7	10200	**113**	31,1	**62**	BC	
14			65,5	**130**	ES	result converted *
19	6200	**69**	49	**97**	ES	result converted *
25			72	**143**	ES	result converted *
27	15000	167	52	**103**	IL	
6	2673	30	8,8	17	NL	
5	875	10	60,6	**120**	RS	
10	9950	**111**	51	**101**	RS	
16	7412,9	**82**	52,83	**105**	RS	
17	9232	**103**	75,85	**150**	RS	
18	14284	**159**	76,9	153	RS	
2	9200	**102**	68	**135**	VT	
3	12000	**133**	50	**99**	VT	
4	na		96	190	VT	
11	20000	222	133	264	VT	
12			102	202	VT	
13	10350	**115**	54	**107**	VT	

* calculation see p. 18

RA*	50-150 %	RA*	50-150 %
Number in RA	**9**	Number in RA	**13**
Percent in RA	**60**	Percent in RA	**68**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

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

Comments:
For the spiking material sample 60% (9) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the food matrix sample A produced with the spiking material sample 68% (13) of the recovery rates were in the range of acceptance.

4.2.2 PCR Results: Mustard (Sinapis alba)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
11	positive	-	negative	-	2/2 (100%)	ASU	
20	positive		negative		2/2 (100%)	ASU	
22	positive	50	negative		2/2 (100%)	ASU	
23a	positive	-	negative	-	2/2 (100%)	ASU	Mustard, yellow
15a	positive	15,5	negative	0	2/2 (100%)	SFA-4p	
15b	positive	45	negative	0	2/2 (100%)	SFA-ID	
17	positive	43,36	negative	< 1	2/2 (100%)	SFA-ID	
18	positive		traces		1/2 (50%)	SFA-ID	
19	positive	> 0,4	negative	< 0,4	2/2 (100%)	SFA-ID	
25	positive		positive		1/2 (50%)	SFA-ID	
3	positive		negative		2/2 (100%)	div	
6	positive		negative		2/2 (100%)	div	
8	positive	90	negative	-	2/2 (100%)	div	
9	positive		negative		2/2 (100%)	div	
11	positive	-	traces	-	1/2 (50%)	div	
23b	negative	-	negative	-	2/2 (100%)	div	Mustard, brow n/black
26	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	16		1	
Number negative	1		14	
Percent positive	94		7	
Percent negative	6		93	
Consensus value	positive		negative	

Methods:
ASU = ASU §64 Methode/method
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 A. The negative result for sample A is plausible, because it is obtained for the detection of brown and black mustard. White/yellow mustard was actually added.
One positive result and two results indicating "traces" were obtained for sample B without any quantitative specification.

Quantitative valuation of results: Sample A

Evaluation number	Mustard	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
11	-		ASU	
20			ASU	
22	50	0,1	ASU	
23a	-		ASU	Mustard, yellow
15a	15,5	-2,7	SFA-4p	
15b	45	-0,3	SFA-ID	
17	43,36	-0,4	SFA-ID	
18			SFA-ID	
19	> 0,4		SFA-ID	
25			SFA-ID	
3			div	
6			div	
8	90	3,4	div	
9			div	
11	-		div	
23b	-		div	Mustard, brow n/black
26			div	

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

Comments:
A kernel density estimation was not done due to the number of < 8 results.

Characteristics: Quantitative evaluation Mustard

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	48,8
Median	45,0
Robust Mean (X)	**48,8**
Robust standard deviation (S*)	**30,3**
Target range:	
Target standard deviation σ_{pt}	**12,2**
lower limit of target range	**24,4**
upper limit of target range	**73,2**
Quotient S/σ_{pt}*	*2,5*
Standard uncertainty $U_{(Xpt)}$	*16,9*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*1,4*
Results in the target range	*3*
Percent in the target range	*60*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a slightly increased variability of results. The quotient S/σ_{pt} was above 2,0. The robust standard deviation is higher than the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited, because there are only a few results at all and for each method. Therefore the target range and the evaluation of results by z-scores has only limited significance. It is given here for orientating information only.*

The robust mean of the evaluation was 98% of the spiking level of mustard to sample A and within the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Mustard" p.39).

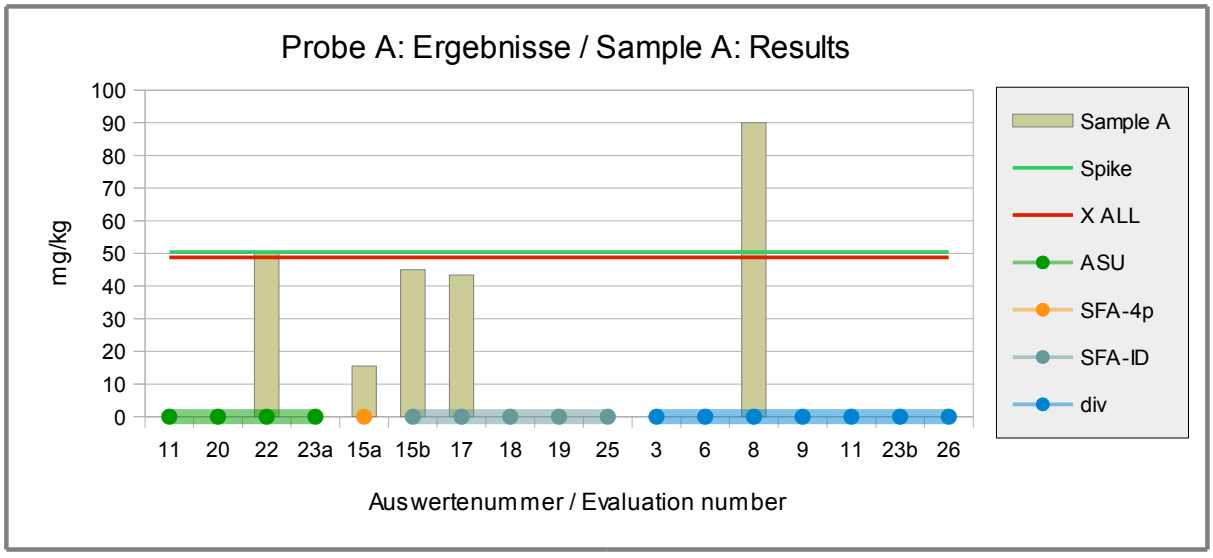


Abb./Fig. 10: PCR Results Mustard
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

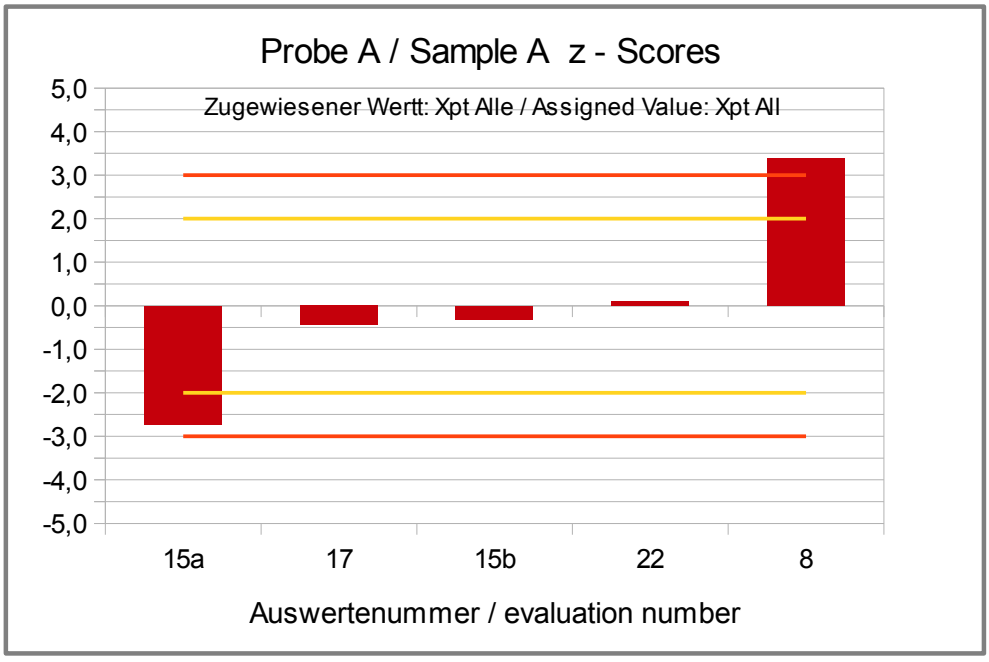


Abb./Fig. 11:
z-Scores (PCR Results Mustard)
Assigned value robust mean of all results

Recovery Rates for Mustard:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
11	-		-		ASU	
20					ASU	
22	13000	**144**	50	**99**	ASU	
23a	-		-		ASU	Mustard, yellow
15a	8520	**95**	15,5	31	SFA-4p	Single results Spike.: 9581/7462 °
15b	s. note		45	**89**	SFA-ID	Single result spike: 281183/180894
17	4477	**50**	43,36	**86**	SFA-ID	
18					SFA-ID	
19	>0,4		> 0,4		SFA-ID	
25					SFA-ID	
3					div	
6					div	
8	57900	643	90	179	div	
9	3385	38			div	
11	-		-		div	
23b	-		-		div	Mustard, brown/black
26					div	

° Mean calculated by DLA

RA*	50-150 %	RA*	50-150 %
Number in RA	**3**	Number in RA	**3**
Percent in RA	**60**	Percent in RA	**60**

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

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
For the spiking material sample 3 participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the food matrix sample A produced with the spiking material sample also 3 recovery rates were in the range of acceptance.

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		
1	positive	52,7	negative	< 2	2/2 (100%)	AQ	
5	positive	78,5	negative	< 2	2/2 (100%)	BC	
7	positive	49,1	negative	< 2	2/2 (100%)	BC	
3	positive	220	negative	< 6.25	2/2 (100%)	BK	
11	positive	150	negative	< 6	2/2 (100%)	BK	
2	positive	15,0	negative	nd	2/2 (100%)	ES	result converted °
4	positive	11,5	negative	< 0,5	2/2 (100%)	ES	result converted °
12	positive	10,2	negative	nd	2/2 (100%)	ES	result converted °
13	positive	11,5	negative	< 0.5	2/2 (100%)	ES	result converted °
14	positive	11,2	negative	< 0,5	2/2 (100%)	ES	result converted °
19	positive	12,0	negative	< 0,5	2/2 (100%)	ES	result converted °
24	positive	15,9	negative	< 0.5	2/2 (100%)	ES	result converted °
25	positive	27,9	negative		2/2 (100%)	ES	result converted °
27	positive	30	negative	< 1	2/2 (100%)	IL	
6	positive	84,5	negative	< 2,50	2/2 (100%)	NL	result converted °
10	positive	398	negative	< 0,24	2/2 (100%)	RS	
16	positive	561,99	negative	< LOD	2/2 (100%)	RS	
17	positive	318,95	negative	< 2.5	2/2 (100%)	RS	

° calculation see p. 18

	Sample A		Sample B	
Number positive	18		0	
Number negative	0		18	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

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

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

Quantitative valuation of results: Sample A

Evaluation number	Sesame	z-Score Xpt_{ALL}	z-Score Xpt_{ES}	Method	Remarks
	[mg/kg]				
1	52,7			AQ	
5	78,5			BC	
7	49,1			BC	
3	220			BK	
11	150			BK	
2	15,0		0,6	ES	result converted °
4	11,5		-0,5	ES	result converted °
12	10,2		-0,9	ES	result converted °
13	11,5		-0,5	ES	result converted °
14	11,2		-0,6	ES	result converted °
19	12,0		-0,3	ES	result converted °
24	15,9		0,9	ES	result converted °
25	27,9		4,5	ES	result converted °
27	30			IL	
6	84,5			NL	result converted °
10	398			RS	
16	561,99			RS	
17	318,95			RS	

° calculation see p. 18

Methoden:

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

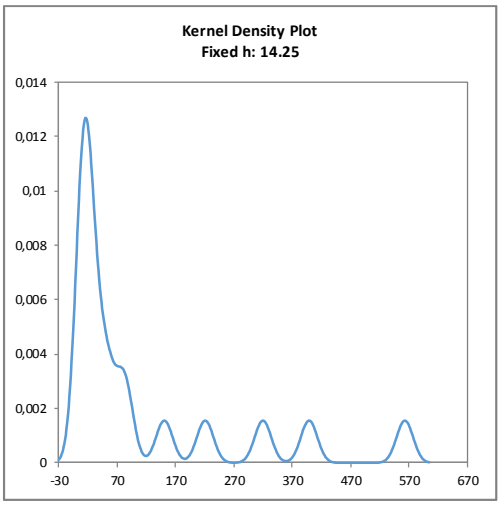


Abb. / Fig. 12:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows only for method ES a nearly normal distribution of results, the few results of the other methods are distributed in a higher range.

Characteristics: Quantitative evaluation Sesame

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method ES** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ ES}}$
Number of results	18	8
Number of outliers	-	1
Mean	114,0	14,4
Median	39,6	11,8
Robust Mean (X)	**75,9**	**13,1**
Robust standard deviation (S*)	**88,1**	**3,06**
Target range:		
Target standard deviation σ_{pt}		**3,3**
lower limit of target range		**6,6**
upper limit of target range		**19,7**
Quotient S/σ_{pt}*		*0,93*
Standard uncertainty $U_{(Xpt)}$		*1,35*
Quotient $U_{(Xpt)}/\sigma_{pt}$		*0,41*
Results in the target range		*7*
Percent in the target range		*88*

Method:
ES = ELISA-Systems

Comments to the statistical characteristics and assigned values:

Due to the strongly differing results a joined evaluation of all results was not done. The evaluation of results from method ES showed a low variability of results. The quotient S/σ_{pt} was clearly below 2,0.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results of method ES is given.

The robust mean of the evaluation of method ES was 17% of the spiking level of sesame to sample A and below the recommendations for the applied method (s. 3.4.3 and "Recovery rates of Sesame" p.44).

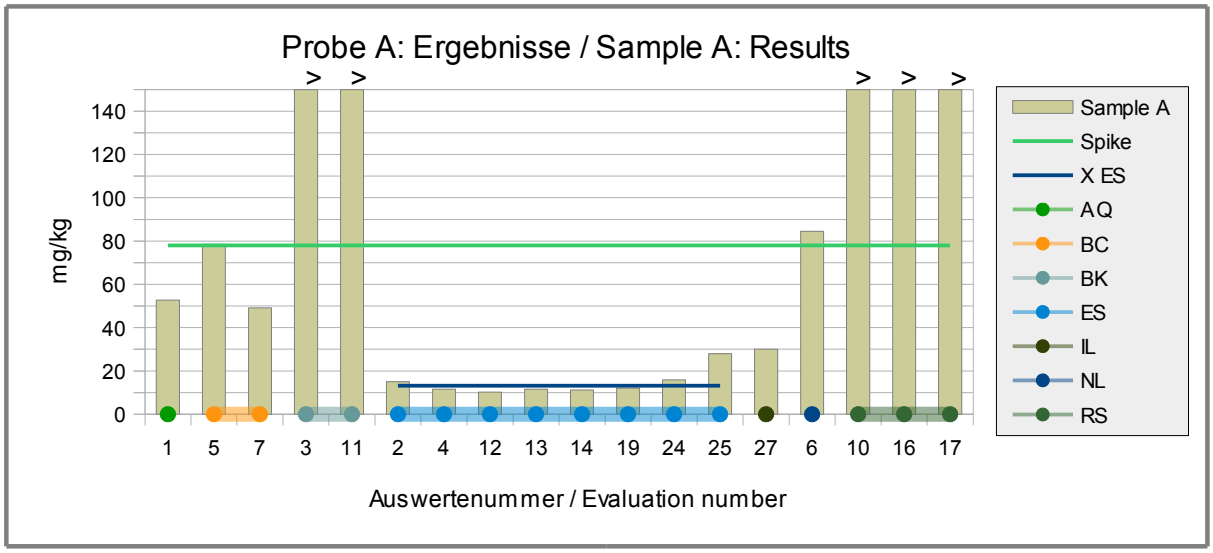


Abb./Fig. 13: ELISA Results Sesame
green line = Spiking level
blue line = Assigned value robust mean results method ES
round symbols = Applied methods (see legend)

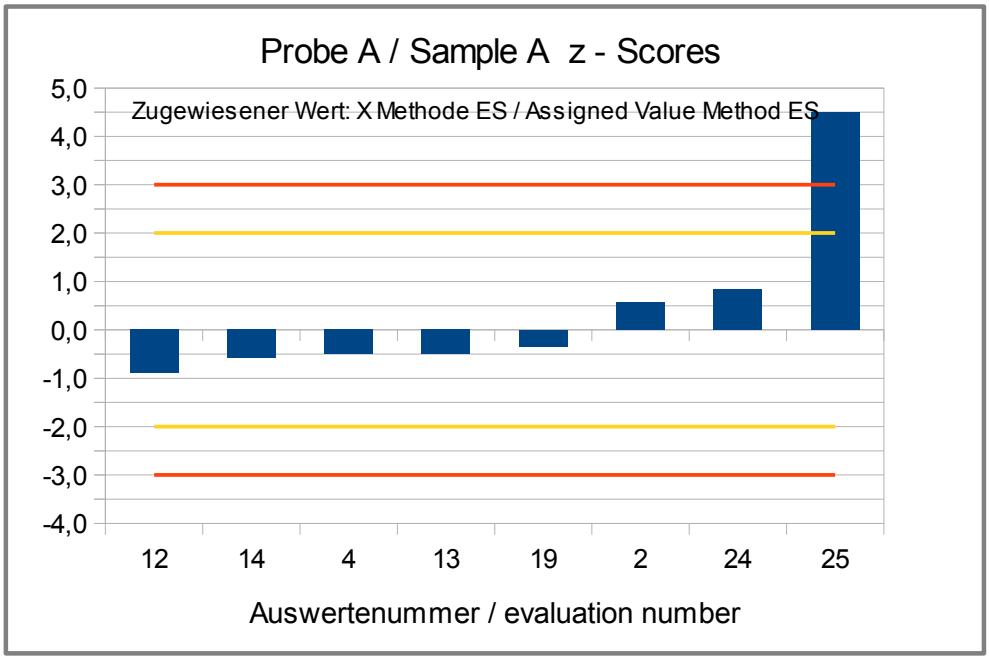


Abb./Fig. 14:
z-Scores (ELISA Results Sesame)
Assigned value robust mean of method ES (ELISA-Systems)

Recovery Rates for Sesame:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	13124	**100**	52,7	**68**	AQ	
5	2960	23	78,5	**101**	BC	
7	12700	**97**	49,1	**63**	BC	
3	59000	450	220	282	BK	
11	28000	214	150	192	BK	
2	2210	17	15,0	19	ES	result converted °
4	na		11,5	15	ES	result converted °
12			10,2	13	ES	result converted °
13	1620	12	11,5	15	ES	result converted °
14			11,2	14	ES	result converted °
19	2230	17	12,0	15	ES	result converted °
24	2080	16	15,9	20	ES	result converted °
25			27,9	36	ES	result converted °
27	10000	76	30	38	IL	
6	5250	40	84,5	**108**	NL	result converted °
10	>200000		398	510	RS	
16	111382	850	561,99	721	RS	
17	51071	390	318,95	409	RS	

° calculation see p. 18

RA*	50-150 %	RA*	50-150 %
Number in RA	**3**	Number in RA	**4**
Percent in RA	**23**	Percent in RA	**22**

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

** Range of acceptance of AOAC for allergen ELISAS

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

Comments:
For the spiking material sample 3 participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the food matrix sample A produced with the spiking material sample 4 of the recovery rates were 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		
25	positive		negative		2/2 (100%)	4L	
6	positive		negative		2/2 (100%)	ASU	
20	positive		positive		2/2 (100%)	ASU	
22	positive	92	negative		2/2 (100%)	ASU	
9	positive		negative		2/2 (100%)	MS	
15a	positive	47,5	negative	0	2/2 (100%)	MS	
21	positive	197	negative		2/2 (100%)	MS	
15b	positive	26,5	negative	0	1/2 (50%)	SFA-ID	
18	positive		negative		2/2 (100%)	SFA-ID	
19	positive	> 0,4	negative	<0,4	1/2 (50%)	SFA-ID	
3	positive		negative		2/2 (100%)	div	
8	positive	40	negative	-	2/2 (100%)	div	
11	positive	-	negative	-	2/2 (100%)	div	
23	positive	-	negative	-	2/2 (100%)	div	
26	positive		negative		1/2 (50%)	div	

	Sample A		Sample B	
Number positive	15		1	
Number negative	0		14	
Percent positive	100		7	
Percent negative	0		93	
Consensus value	positive		negative	

Methods:

4L = 4LAB Diagnostics

ASU = ASU §64 Methode/method

MS = Microsynth

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

div = not indicated / other method

Comments:

The consensus values are in agreement with the spiking of sample A.
One positive result for sample B was submitted without quantitative specification.

Quantitative valuation of results: Sample A

Evaluation number	Sesame	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
25			4L	
6			ASU	
20			ASU	
22	92	0,9	ASU	
9			MS	
15a	47,5	-1,5	MS	
21	197	6,5	MS	
15b	26,5	-2,6	SFA-ID	
18			SFA-ID	
19	> 0,4		SFA-ID	
3			div	
8	40	-1,9	div	
11	-		div	
23	-		div	
26			div	

Methods:

4L = 4LAB Diagnostics

ASU = ASU §64 Methode/method

MS = Microsynth

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

div = not indicated / other method

Comments:

A kernel density estimation was not done due to the number of < 8 results.

Characteristics: Quantitative evaluation Sesame

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	5
Number of outliers	0
Mean	80,6
Median	47,5
Robust Mean (X)	**75,4**
Robust standard deviation (S*)	**66,6**
Target range:	
Target standard deviation σ_{pt}	**18,8**
lower limit of target range	**37,7**
upper limit of target range	**113**
Quotient S/σ_{pt}*	*3,5*
Standard uncertainty $U_{(Xpt)}$	*37,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*2,0*
Results in the target range	*3*
Percent in the target range	*60*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed an increased variability of results. The quotient S/σ_{pt} was above 2,0. The robust standard deviation is higher than the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited, because there are only a few results at all and for each method. Therefore the target range and the evaluation of results by z-scores has only limited significance. It is given here for orientating information only.*

The robust mean of the evaluation was 97% of the spiking level of sesame to sample A and within the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Sesame" p.49).

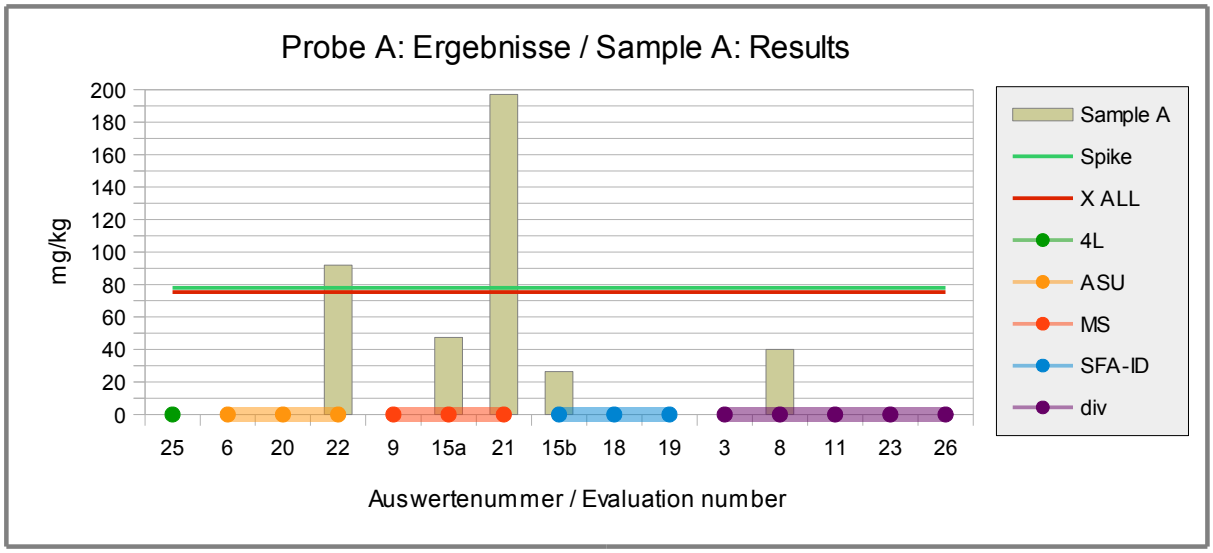


Abb./Fig. 15: PCR Results Sesame
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

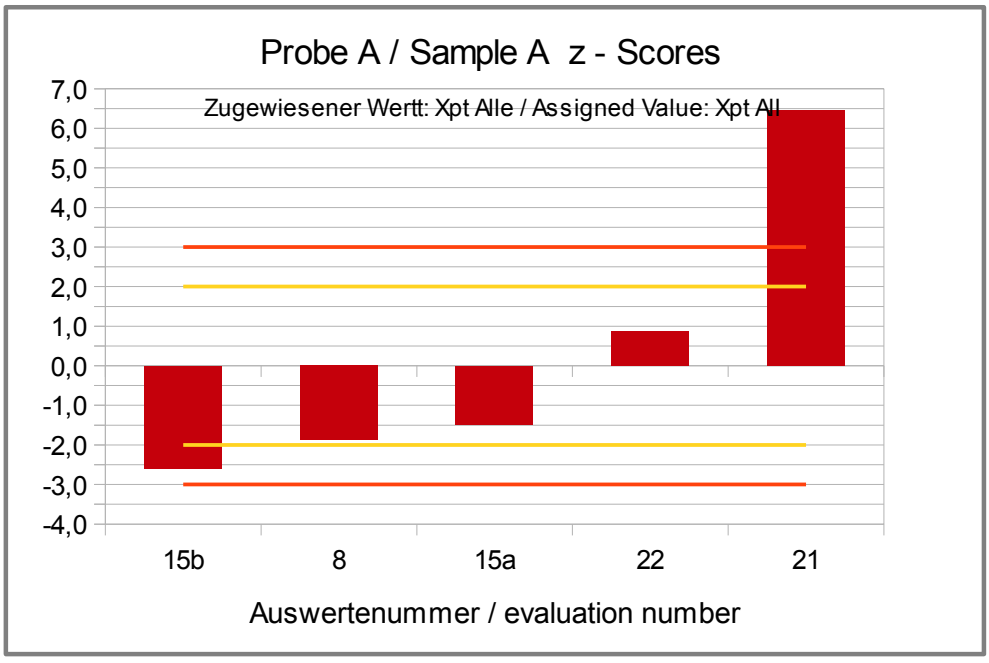


Abb./Fig. 16:
z-Scores (PCR Results Sesame)
Assigned value robust mean of all results

Recovery Rates for Sesame:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
25					4L	
6					ASU	
20					ASU	
22	31000	237	92	**118**	ASU	
9					MS	
15a	17600	**134**	47,5	**61**	MS	Single results Spike: 20232/14971 °
21	8828	**67**	197	253	MS	
15b	8860	**68**	26,5	34	SFA-ID	Single results Spike: 10455/7266 °
18					SFA-ID	
19	>0,4		> 0,4		SFA-ID	
3					div	
8	48300	**369**	40	**51**	div	
11	-		-		div	
23	-		-		div	
26					div	

° Mean calculated by DLA

RA*	50-150 %	RA*	50-150 %
Number in RA	3	Number in RA	3
Percent in RA	60	Percent in RA	60

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
4L = 4LAB Diagnostics
ASU = ASU §64 Methode/method
MS = Microsynth
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the spiking material sample 3 participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the food matrix sample A produced with the spiking material sample also 3 recovery rates were in the range of acceptance.

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Celery

none

5.1.2 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	1	05.09.16	positive	40,4	negative	<2	positive	26081	Mustard	AgraQuant Mustard
AQ	24	06.09.16	positive	48,55	negative	<2	positive	5946,05	Mustard	AgraQuant Mustard (CO-KAL2148), RomerLabs
BC	7	05.09.16	positive	31,1	negative	<2	-	10200	Mustard	Biocheck Mustard Check
ES	14	28.09.16	-	19,65	-	< 1	-		Mustardprotein	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
ES	19	22.09.16	positive	14,7	negative	<1	positive	1860	Mustardprotein	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
ES	25	31.08.16	positive	21,6	negative		-		Mustardprotein	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
IL	27	10.08.16	positive	52	negative	< 1	positive	15000	Mustard	Immunolab Mustard ELI-SA
NL	6	02.09.16	positive	8,8	negative	< 2,50	positive	2673	Mustard	nutriLinia Mustard (NC-6008), Transia
RS	5	03.10.16	positive	60,6	positive	0,88	positive	875	Mustard	Ridascreen Fast Senf / Mustard (R6152), r-Bio-pharm
RS	10	21-22/09/2016	positive	51	positive	1,79	positive	9950	Mustard	Ridascreen Fast Senf / Mustard (R6152), r-Bio-pharm
RS	16	28.09.16	-	52,83	-	0,833	-	7412,9	Mustard	Ridascreen Fast Senf / Mustard (R6152), r-Bio-pharm
RS	17	19.08.16	positive	75,85	negative	<0.5	positive	9232	Mustard	Ridascreen Fast Senf / Mustard (R6152), r-Bio-pharm
RS	18	19.09.	positive	76,9	positive	0,91	positive	14284	Mustardpowder	Ridascreen Fast Senf / Mustard (R6152), r-Bio-pharm
VT	2	04.10.16	positive	68	negative	nd	positive	9200	Mustard	Veratox Mustard Allergen, Neogen
VT	3		positive	50	negative	<2.5	positive	12000	Mustard	Veratox Mustard Allergen, Neogen
VT	4	06.09.16	-	96	-	<2,5	-	na	Mustard	Veratox Mustard Allergen, Neogen
VT	11	17.08.16	positive	133	positive	<2,5	positive	20000	Mustard	Veratox Mustard Allergen, Neogen
VT	12	16.09.16	positive	102	negative	nd	-		Mustard	Veratox Mustard Allergen, Neogen
VT	13	10.03.16	positive	54	negative	<2.5	positive	10350	Mustard	Veratox Mustard Allergen, Neogen

continued ELISA Mustard:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	1			
AQ	24	-	-	-
BC	7			
ES	14			
ES	19			
ES	25			
IL	27			
NL	6		As Per Kit Instructions	
RS	5		1g + 20ml extraction buffer 60ºC	
RS	10	Mustard	According to Manual	
RS	16	specific	1 g sample + 20 ml extraction buffer from kit, 10 minutes at 60°C, centrifugation	
RS	17		As Per Kit Instructions	
RS	18		According to Manual	
VT	2		kit extraction buffer / extracted for 15 min/ 60°C	single results
VT	3			
VT	4	mustard	As Per Kit Instructions	
VT	11	Mustardprotein from seeds of white mustard (Sinapis alba), black mustard (Brassica nigra) and brown mustard (Brassica juncea)	As Per Kit Instructions	
VT	12		Extraction:60C pre-heated TRIS-EDTA / 15 min @ 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	spiking material was not tested
VT	13		As Per Kit Instructions	

5.1.3 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	1	23.08.16	positive	52,7	negative	<2	positive	13124	Sesame	AgraQuant Sesame
BC	5	03.10.16	positive	78,5	negative	<2	positive	2960	Sesame	Biocheck (UK) Sesame
BC	7	30.09.16	positive	49,1	negative	<2	-	12700	Sesame	Biocheck Sesame Check
BK	3	23.08./30.09. / 27.09.	positive	220	negative	<6.25	positive	59000	Sesame	BioKits, Sesame Assay Kit (902070X), Neogen
BK	11	15.08.16	positive	150	negative	<6	positive	28000	Sesame	BioKits, Sesame Assay Kit (902070X), Neogen
ES	2	07.10.16	positive	3,4	negative	nd	positive	500	Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	4	06.09.16	-	2,6	-	<0,5	-	na	Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	12	18.08.16	positive	2,3	negative	nd	-		Sesame seed protein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	13	10.05.16	positive	2,6	negative	<0.5	positive	366,3	Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	14	28.09.16	-	2,54	-	< 0,5	-		Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	19	22.09.16	positive	2,7	negative	<0,5	positive	505	Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	24	31.08.16	positive	3,6	negative	<0.5	positive	470,5	Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
ES	25	31.08.16	positive	6,3	negative		-		Sesameprotein	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
IL	27	10.08.16	positive	30	negative	< 1	positive	10000	Sesame	Immunolab Sesame ELISA
NL	6	28.09.16	positive	19,1	negative	< 2,50	positive	1185,9	Sesameprotein	nutriLinia Sesam (NC-6006), Transia
RS	10	26-27/09/2016	positive	398	negative	<0,24	positive	>200000	Sesame	Ridascreen Fast Sesame (R7202), r-biopharm
RS	16	28.09.16	-	561,99	-	< LOD	-	111382	Sesame	Ridascreen Fast Sesame (R7202), r-Biopharm
RS	17	24.08.16	positive	318,95	negative	<2.5	positive	51071	Sesame	other: please fill in!

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	1			
BC	5		1g + 20ml extraction buffer 60ºC	
BC	7			
BK	3			
BK	11	Sesame proteins	As Per Kit Instructions	
ES	2		kit extraction buffer / extracted for 15 min/ 60°C	single results
ES	4	sesame	as described by the manufacturer	
ES	12	anti-sesame seed 2S albumin anitbodies	Extraction: room temperature PBS (pH adjustmen if necessary) / 15 min @ 60C in shaking w aterbath / centrifugation Determination: 4 parameter curve	spiking material w as not tested
ES	13	2S-albumin	as per kit instructions	
ES	14			
ES	19			
ES	24	-	-	-
ES	25			
IL	27			
NL	6		As Per Kit Instructions	
RS	10	Sesame	According to Manual	
RS	16	specific	1 g sample + 20 ml extraction buffer from kit, 10 minutes at 60°C, centrifugation	
RS	17		As Per Kit Instructions	R- Biopharm FAST Sesame

5.1.4 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
4L	25	23.08.16	positive		positive		-		Celery-DNA	4 LAB
ASU	6	04.09.16	positive		positive		positive			ASU §64 L 08.00-56
ASU	11	12.08.16	positive	-	negative	-	positive	-	Celery-DNA	ASU §64 L 08.00-56
ASU	20	16.9.	+		-		+		given as	ASU §64 L 08.00-56
ASU	22	15.09.16	positive	30	positive	< 20	positive	8400	Celery seed, dried	ASU §64 L 08.00-56
ASU	23	08.09.16	positive	--	positive	--	positive	--	given as	ASU §64 L 08.00-56
ASU	26	24.08.	positive		negative		positive		Celery-DNA	ASU §64 L 08.00-56
FP	14	15.09.16	-	10,18	-	3,42	-		Celery	Foodproof Celery Detection Kit - 5`Nuclease
MS	9		positive		negative		positive		Celery-DNA	Microsynth
MS	15b	20.09.16	positive	9,5	positive	15,5	positive	8948/8403	Allergen/Food	Microsynth AllAlla
SFA-4p	15a	14.09.16	positive	16	positive	4,5	positive	6860/6137	Allergen/Food	Sure Food Allergen 4plex, Congen / r-Biopharm
SFA-ID	17	15.08.16	positive	99,06	positive	23,32	positive	19397	Celery	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	18	31.08.16	positive		positive		positive		Celery-DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	19	22.09.16	positive	>0,4	positive	>0,4	positive	>0,4	Celery-DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-Q	10	13-14/9/2016	positive	14,75	positive	7,5	positive	5223	Celery	Sure Food Allergen QUANT, Congen / r-Biopharm
SFA-Q	21	12.09.16	positive	9,9	positive	1,1	positive	7597	Celery	Sure Food Allergen QUANT, Congen / r-Biopharm
div	1	06.10.16	negative		positive		positive		Celery DNA	In house method
div	3	29.09.	positive		negative		positive		Celery-DNA	realtime PCR-method
div	5	03.10.16	negative	<1	negative	<1	positive	>1	Celery	in-house developed
div	8	05.10.16	positive	150	positive	140	positive	104200	Celery	ApiumMat3

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
4L	25			
ASU	6			
ASU	11		CTAB / Protease K/ Amylase / Chloroform + Promega Wizard/ Realtime PCR/ - / 45 cycles	
ASU	20			
ASU	22		CTAB-precipitation method according to ASU § 64 L 18.00-22	LOD appr. 5 mg/kg, LOQ 20 mg/kg
ASU	23	Mannitol dehydrogenase gene	CTAB	
ASU	26	Mannitol-Dehydrogenase	CTAB precipitation, QIAgen PCR Purification Kit, Real Time PCR	
FP	14		Real Time PCR	
MS	9	Celery-DNA	Macherey Nagel Nucleo Spin Food with optimization: increased sample weight, buffer change (wash with lysis buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination step with UNG; own thermoprofile	
MS	15b		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 45 Cyclen	Mean from two analysis / spiking sample undiluted
SFA-4p	15a		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 35 Cyclen	Mean from two analysis / spiking sample undiluted
SFA-ID	17		As Per Kit Instructions	
SFA-ID	18		DNA-Isolation with SureFood PREP Advanced, Congen/R-Biopharm	
SFA-ID	19			
SFA-Q	10	Celery	Real time PCR	
SFA-Q	21	Celery	Kit SureFood PREP Advanced, S1053, PCR according to kit instructions, 45 cycles	
div	1		gel electrophoresis, LOD 10ppm	
div	3			
div	5	MTD	Tris DNA Extraction, Column clean-up,Taqman reagents real-time PCR detection	
div	8		CTAB, Magnetiv Beads, M&N-columns	

5.1.5 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
ASU	11	12.08.16	positive	-	negative	-	positive	-	Mustard-DNA	ASU §64 L 08.00-59
ASU	20	10.8.	+		-		+		given as	ASU §64 L 08.00-59
ASU	22	15.09.16	positive	50	negative		positive	13000	Mustardsaat, white	ASU §64 L 08.00-59
ASU	23a	08.09.16	positive	--	negative	--	positive	--	given as	ASU §64 L 08.00-59
SFA-4p	15a	14.09.16	positive	15,5	negative	0	positive	9581/7462	Allergen/Food	Sure Food Allergen 4plex, Congen / r-Biopharm
SFA-ID	15b	29.09.16	positive	45	negative	0	positive	281183/180894	Allergen/Food	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	17	15.08.16	positive	43,36	negative	<1	positive	4477	Mustard	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	18	31.08.16	positive		in Spuren		positive		Mustard-DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	19	22.09.16	positive	>0,4	negative	<0,4	positive	>0,4	Mustard-DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	25	23.08.16	positive		positive		-		Mustard-DNA	Sure Food Allergen ID, Congen / r-Biopharm
div	3	29.09.	positive		negative		positive		Mustard-DNA	realtime PCR-Verfahren
div	6	05.09.16	positive		negative		positive			Method internal
div	8	05.10.16	positive	90	negative	-	positive	57900	Mustard	SinAlba
div	9		positive		negative		positive	3385	Mustard-DNA	Primer+Probes: Microsynth; Method: Fuchs et al. J. Agric. Food Chem. 2010, 58, 11193–11200 Palle-Reisch et al. Food Chemistry 138 (2013) 348–355
div	11	12.08.16	positive	-	traces at LOD	-	positive	-	Mustard-DNA and Brassica Species	internal method: realtime PCR 45 cycles
div	23b	12.09.16	negative	--	negative	--	negative	--		Palle-Reisch, Food Chemistry 153 (2014) 66–73
div	26	18.08.	positive		negative		positive		Mustard-DNA	Mustorp et al. 2008 Eur Food Res Technol. 226: 771-778

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	11	white mustard (Sinapis alba)	CTAB / Protease K/ Amylase / Chloroform + Promega Wizard/ Realtime PCR/ - / 45 Cycles	
ASU	20			
ASU	22		CTAB-precipitation method according to ASU § 64 L 18.00-22	LOD appr. 5 mg/kg
ASU	23a	mRNAforMADSD	CTAB	
SFA-4p	15a		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 35 Cycles	Mean from two analysis / spiking sample undiluted
SFA-ID	15b		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 35 Cycles	Mean from two analysis / spiking sample undiluted
SFA-ID	17		As Per Kit Instructions	
SFA-ID	18		DNA-Isolation mit SureFood PREP Advanced, Congen/R-Biopharm	
SFA-ID	19			
SFA-ID	25			
div	3			
div	6			
div	8		CTAB, Magnetiv Beads, M&N-column	
div	9	Sinapis alba, Brassica juncea, Brassica nigra	Macherey Nagel Nucleo Spin Food with optimization: increased sample weight, buffer change (wash with lysis buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination step with UNG; own thermoprofile	no distinction between Brassica juncea and nigra, quantification related to Sinapis alba; Brassica juncea/nigra present in traces
div	11	white mustard (Sinapis alba), black mustard (Brassica nigra) and brown mustard (Brassica juncea)	CTAB / Protease K / Amylase / Chloroform + Promega Wizard/ Realtime PCR/ - / 45 Cycles	
div	23b	AJ415649	CTAB	
div	26	major allergen sin a1	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	

5.1.6 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
4L	25	23.08.16	positive		negative				Sesame-DNA	4 LAB
ASU	6	05.09.16	positive		negative		positive			ASU L 18.00.19:2014 mod
ASU	20	10.8.	+		+		+		given as	ASU §64 L 18.00-19
ASU	22	16.09.16	positive	92	negative		positive	31000	Sesame	ASU §64 L 18.00-19
MS	9		positive		negative		positive		Sesame-DNA	Microsynth
MS	15a	28.09.16	positive	47,5	negative	0	positive	20232/14971	Allergen/Food	Microsynth AlAlIB
MS	21	19.09.16	positive	197	negative		positive	8828	Sesame	AlAlIB, microsynth
SFA-ID	15b	27.09.16	positive	26,5	negative	0	positive	10455/7266	Allergen/Food	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	18	16.08.	positive		negative		positive		Sesame-DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	19	22.09.16	positive	>0,4	negative	<0,4	positive	>0,4	Sesame-DNA	Sure Food Allergen ID, Congen / r-Biopharm
div	3	29.09.	positive		negative		positive		Sesame-DNA	realtime PCR-method
div	8	05.10.16	positive	40	negative	-	positive	48300	Sesame	Ses-Cy5
div	11	12.08.16	positive	-	negative	-	positive	-	Sesame-DNA	internal Method: realtime PCR 45 Cycles
div	23	08.09.16	positive	--	negative	--	positive	--	given as	I. Laube 2007
div	26	18.08.	positive		negative		positive		Sesame-DNA	Sure Food Allergen , Congen / r-Biopharm

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
4L	25			
ASU	6			
ASU	20			
ASU	22		CTAB-Precipitation method according to ASU § 64 L 18.00-22	LOD appr. 5 mg/kg
MS	9	Sesame-DNA	Macherey Nagel Nucleo Spin Food with optimization: increased sample weight, buffer change (wash with lysis buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination step with UNG; own thermoprofile	
MS	15a		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 45 Cycles	Mean from two analysis / spiking sample undiluted
MS	21		Kit SureFood PREP Advanced, Quantifast Mastermix QIAGEN, 45 Zyklen	
SFA-ID	15b		CTAB Isolation / Prot.K / QIAquick Purification Kit / RT-PCR / 35 Cycles	Mean from two analysis / spiking sample undiluted
SFA-ID	18		DNA-Isolation with SureFood PREP Advanced, Congen/R-Biopharm	
SFA-ID	19			
div	3			
div	8		CTAB, Magnetiv Beads, M&N-columns	
div	11		CTAB / Protease K / Amylase / Chloroform + Promega Wizard/ Realtime PCR/ - / 45 Cycles	
div	23	Sesamum indicum omega-6 fatty acid desaturase	CTAB	
div	26	unknown	CTAB Präzipitation, QIAgen PCR Purification Kit, Real Time PCR	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 04-2016 Sample A

Weight whole sample	3,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,22	66	25,3
2	5,04	68	27,0
3	5,14	68	26,5
4	5,23	52	19,9
5	5,21	56	21,5
6	5,04	60	23,8
7	5,00	54	21,6
8	5,19	63	24,3
9	5,13	62	24,2
10	5,01	70	27,9

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
Mean	61,9	Partikel
Standard deviation	6,66	Partikel
χ^2 (CHI-Quadrat)	6,45	
Probability	**69**	%
Recovery rate	110	%

Normal distribution		
Number of samples	10	
Mean	24,2	mg/kg
Standard deviation	2,60	mg/kg
rel. Standard deviaton	10,8	%
Horwitz standard deviation	9,9	%
HorRat-value	**1,1**	
Recovery rate	110	%

Microtracer Homogeneity Test
DLA 04-2016 Spiking Material Sample

Weight whole sample	1,40	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	78	31,0
2	5,27	82	31,1
3	4,96	68	27,4
4	5,09	71	27,9
5	5,07	75	29,6
6	5,31	84	31,6
7	5,25	79	30,1
8	5,03	71	28,2
9	5,16	67	26,0
10	4,99	73	29,3

Poisson distribution		
Number of samples	10	
Degree of freedom	9	
Mean	74,8	Partikel
Standard deviation	4,66	Partikel
χ^2 (CHI-Quadrat)	2,62	
Probability	**98**	%
Recovery rate	100	%

Normal distribution		
Number of samples	10	
Mean	29,2	mg/kg
Standard deviation	1,82	mg/kg
rel. Standard deviaton	6,2	%
Horwitz standard deviation	9,6	%
HorRat-value	**0,6**	
Recovery rate	100	%

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		SWITZERLAND
		Germany
		SWITZERLAND
		CANADA
		CANADA
		ITALY
		Germany
		Germany
		ZYPRUS
		Germany
		ITALY
		GREAT BRITAIN
		Germany
		GREAT BRITAIN
		Germany
		Germany
		Germany
		AUSTRIA
		GREAT BRITAIN
		Germany
		Germany
		AUSTRIA
		GREAT BRITAIN
		CANADA
		Germany
		CANADA
		Germany

[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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