Proficiency Tests
DLA
food
cosmetics
consumer goods
www.dla-lvu.de

Evaluation Report
proficiency test

DLA 01/2017

Allergens I:

Milk (Casein) and Soya

in Sausage

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

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

Coordinator of this PT:
Dr. Matthias Besler

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

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA 01/2017
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (15 May 2017) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler (Technischer Leiter / Technical Manager) - *gezeichnet / signed M. Besler* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - *gezeichnet / signed G. Wichmann* Datum / Date: 15 May 2017
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.

Inhalt / Content

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 milk and soya, 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 500 µm).
The samples A and B were portioned after homogenization to approximately 25 g in plastic bags, evacuated and shrink-wrapped. Afterwards the samples were heated for 1h at 100°C.
For the spiking level sample, the allergenic compounds milk and soya were added during a multi-stage addition of potato flour and homogenization. Afterwards the whole sample was sieved by means of a centrifugal mill (mesh 500 µm) and portioned to approximately 10 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	96,0 g/100 g	-
Potato flour Ingredients: Potato, E471, E304, E223, E100	-	3,96 g/100 g	99,8 g/100 g
Colouring agent E120	-	0,010 g/100 g	-
Milk: - as skimmed milk powder* - thereof 37% total protein** - thereof casein***	-	 113 mg/kg 41,6 mg/kg 33,3 mg/kg	 103 mg/kg 37,9 mg/kg 30,3 mg/kg
Soya: - as Soy flour* - thereof 37% total protein**	-	 226 mg/kg 83,4 mg/kg	 101 mg/kg 37,3 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	< 0,4 g/100 g	< 0,2 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl)
*** Protein contents according to literature values (approx. 80% casein in total milk protein)[32]

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples showed a probability of 20%. 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 1,3 respectively. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis. 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 usually regarded by DLA as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [16, 17, 20, 21]. This criterion is fulfilled for sample B with a Ss of 20,5% with the ELISA test for casein/milk (AgraQuant)(see page 7). For estimating the homogeneity it should be considered, that the processing of the samples made the analysis more difficult (see recovery rates). For this reason only the above-mentioned ELISA results were available as part of the homogeneity testing.

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

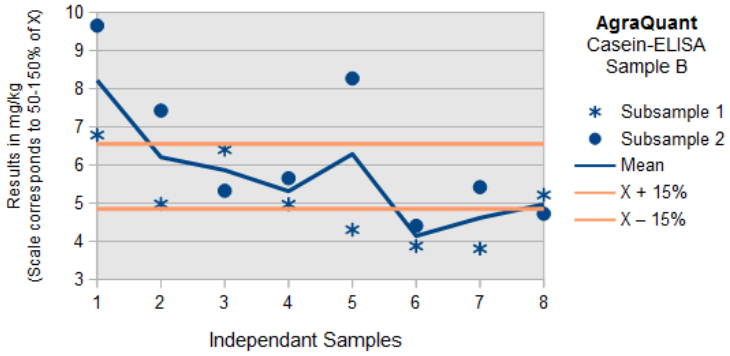
ELISA-Tests: Homogenität Milch / Homogeneity Milk

Romerlabs AgraQuant Casein

Sample weights: 0,50 g (0,45 – 0,55 g)
Number of replicates: 2
Overall result: as Skimmed milk powder 5,70 ± 1,17 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,78	9,65	8,22
2	4,98	7,43	6,20
3	6,40	5,32	5,86
4	4,97	5,65	5,31
5	4,30	8,27	6,28
6	3,87	4,40	4,14
7	3,81	5,42	4,61
8	5,21	4,72	4,97

General average X	5,70	
SD of sample means Sx	1,27	22,3%
SD within-samples Sw	1,47	25,8%
SD between-samples Ss	1,17	20,5%



2.1.2 Stability

The sample material is a sausage meat, which was heated to 100°C for 1h after production and bottling into vacuum bags. 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 2nd week of 2017. The testing method was optional. The tests should be finished at March 3rd 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 milk (casein) and/or soya 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 (potato powder/maltodextrin) in similar amounts without further processing.

Please note the attached information on the proficiency test.
(see documentation, section 5.4 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.
13 out of 14 registered participants submitted at least one result.

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-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} [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. 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} [29-31]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soya flour	Boiled sausage (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled sausage (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59

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 4 and 5, 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 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

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 for milk were evaluated as milk protein. Thus the results were converted considering the literature and test kit values approx. 27,0% protein (full cream milk powder) or 35,1% protein (skimmed milk powder) (AgraQuant, Veratox).
For casein all present results were submitted as casein(s), thus no recalculation was necessary.

ELISA-results for soya were evaluated as soy protein. Thus the results given as soy flour were converted considering the literature and test kit values (approx. 47,0% protein, Veratox).

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 Milk

4.1.1 ELISA Results: Milk (as milk protein)

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	0,13	positive	1,79	1/2 (50%)	AQ	result converted °
6	negative	<2,5	positive	14,9	2/2 (100%)	RS-F	
8	negative	<2,5	positive	6,99	2/2 (100%)	RS-F	
10	negative	<2,5	positive	7,20	2/2 (100%)	RS-F	
11	negative	<2,5	positive	18,8	2/2 (100%)	RS-F	
12	negative	< LOD	positive	10,3	2/2 (100%)	RS-F	
13	negative	<2,5	positive	7,33	2/2 (100%)	RS-F	
9	negative		positive	2,56	2/2 (100%)	VT	result converted °

° Conversion p. 18

	Sample A		Sample B	
Number positive	1		8	
Number negative	7		0	
Percent positive	13		100	
Percent negative	88		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

The consensus values are in agreement with the spiking of sample B. One positive result for sample A was obtained near the limit of determination.

Quantitative valuation of results: Sample B

Evaluation number	Milk protein	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
1	1,79	-1,9		AQ	Result converted °
6	14,9	1,7	1,5	RS-F	
8	6,99	-0,5	-1,4	RS-F	
10	7,20	-0,4	-1,4	RS-F	
11	18,8	2,8	2,9	RS-F	
12	10,3	0,5	-0,2	RS-F	
13	7,33	-0,4	-1,3	RS-F	
9	2,56	-1,7		VT	Result converted °

° Conversion p. 18

Methods:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

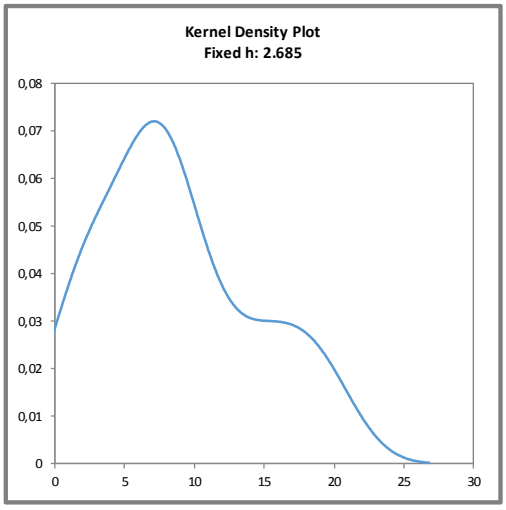


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 with a shoulder at > 12 mg/kg due to two high results obtained by method RS-F.

Characteristics: Quantitative evaluation Milk (as milk protein)

Sample B

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	8	6
Number of outliers	0	0
Mean	8,74	10,9
Median	7,27	8,83
Robust Mean (X)	**8,68**	**10,9**
Robust standard deviation (S*)	**6,45**	**5,58**
Target range:		
Target standard deviation σ_{pt}	**3,58**	**2,73**
lower limit of target range	**1,51**	**5,47**
upper limit of target range	**15,8**	**16,4**
Quotient S/σ_{pt*} or σ_{pt}*	*1,8*	*2,0*
Standard uncertainty $U_{(Xpt)}$	*2,85*	*2,85*
Quotient $U_{(Xpt)}/\sigma_{pt*}$ or σ_{pt}	*0,80*	*1,0*
Results in the target range	*7*	*5*
Percent in the target range	*88*	*83*

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

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences.

The evaluation of results of all methods showed an increased variability of results. The quotient S/σ_{pt} was 3,0. Thus the evaluation was performed considering the standard uncertainty by z´-scores. The quotient S*/σ_{pt}` was then below 2,0.*

The evaluation of the results from method RS-F showed a normal variability of results. The quotient S/σ_{pt} was 2,0.*

The robust standard deviations are 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 21% and 26% of the spiking level of milk to sample B and thus below the recommendations for the applied methods (s. 3.4.3 and "recovery rates for milk protein", see page 29).

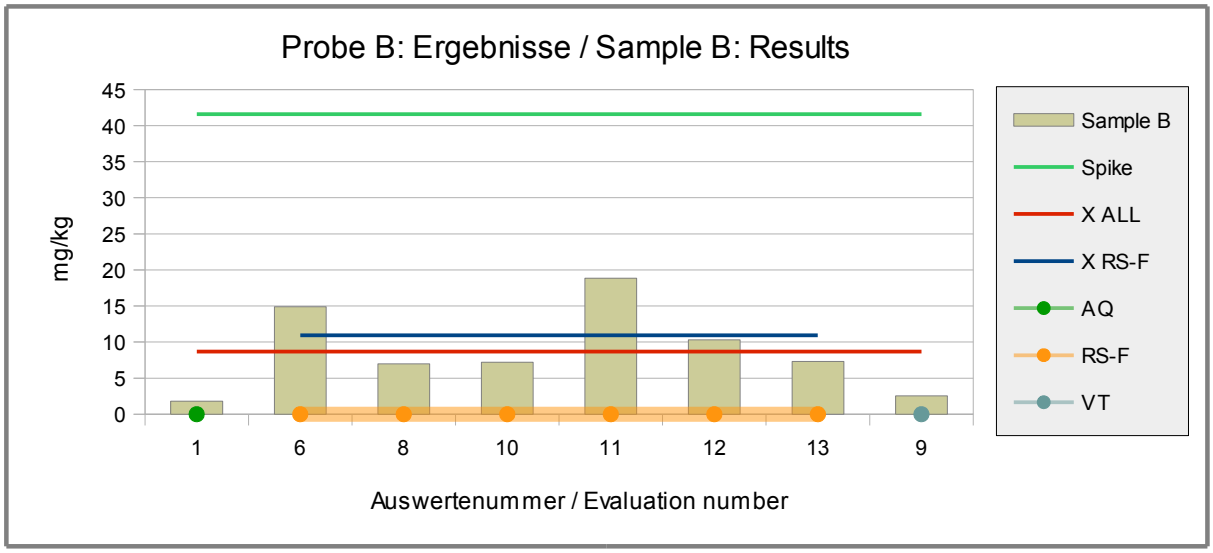


Abb./Fig. 2: ELISA Results Milk (as milk protein)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

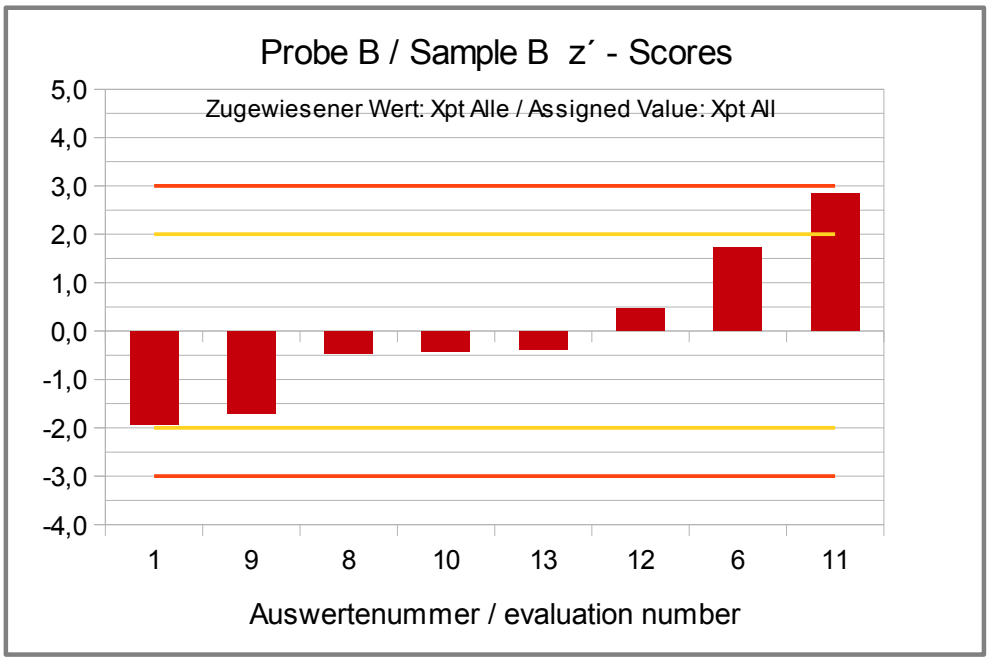


Abb./Fig. 3:
z'-Scores (ELISA Results as milk protein) Assigned value robust mean of all results

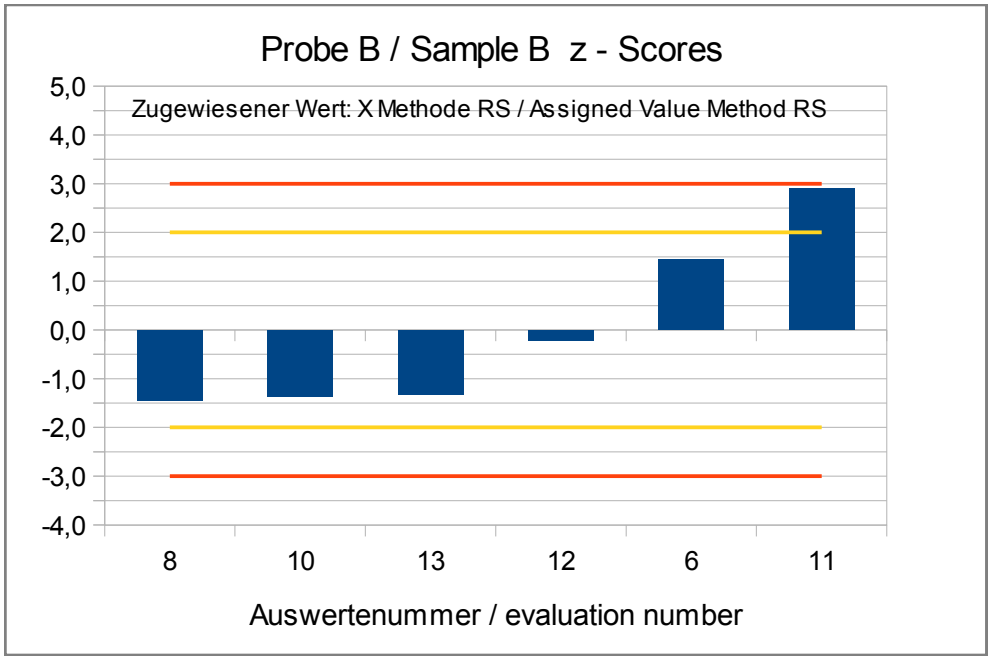
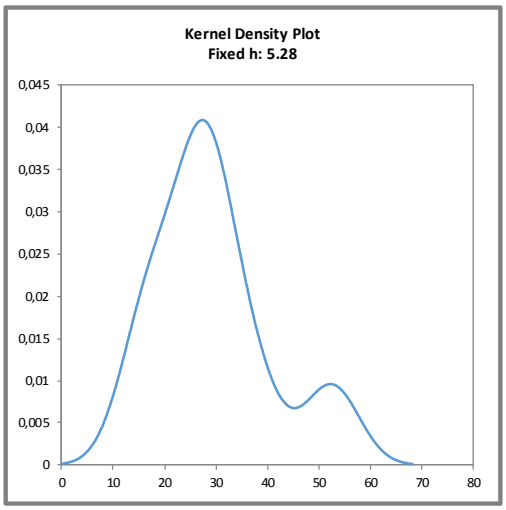


Abb./Fig. 4:
z-Scores (ELISA Results as milk protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Milk protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
1	52,4	3,4		AQ	Result converted °
6	25,0	-0,5	-0,1	RS-F	
8	27,8	-0,1	0,4	RS-F	
10	28,6	0,1	0,5	RS-F	
11	15,7	-1,8	-1,5	RS-F	
12	18,4	-1,4	-1,1	RS-F	
13	37,0	1,2	1,8	RS-F	
9	29,3	0,2		VT	Result converted °

° Conversion p. 18



Methods:

AQ = AgraQuant, RomerLabs

RS-F= 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 side peak at approx. 50 mg/kg, which can be assigned to a single value (method AQ).

Characteristics: Quantitative evaluation Milk (as milk protein)

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	8	6
Number of outliers	0	0
Mean	29,3	25,4
Median	28,2	26,4
Robust Mean (X)	**28,2**	**25,4**
Robust standard deviation (S*)	**10,3**	**8,67**
Target range:		
Target standard deviation σ_{pt}	**7,04**	**6,35**
lower limit of target range	**14,1**	**12,7**
upper limit of target range	**42,3**	**38,1**
Quotient S/σ_{pt}*	*1,5*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*4,56*	*4,43*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,65*	*0,70*
Results in the target range	*7*	*6*
Percent in the target range	*88*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences (one high single value).
The evaluation of results of all methods and of results of method RS-F showed a normal variability with quotients S/σ_{pt} 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 74% and 67% of the spiking level of milk to the spiking level sample and thus in the range of the recommendations for the applied methods (s. 3.4.3 and "recovery rates for milk protein", see page 29).

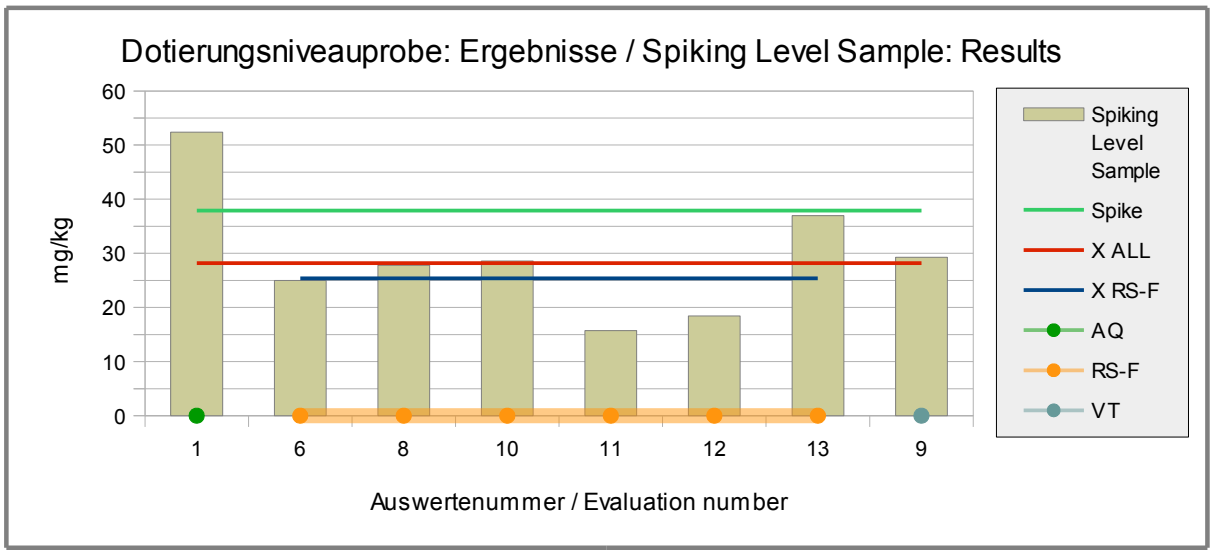


Abb./Fig. 6: ELISA Results Milk (milk protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

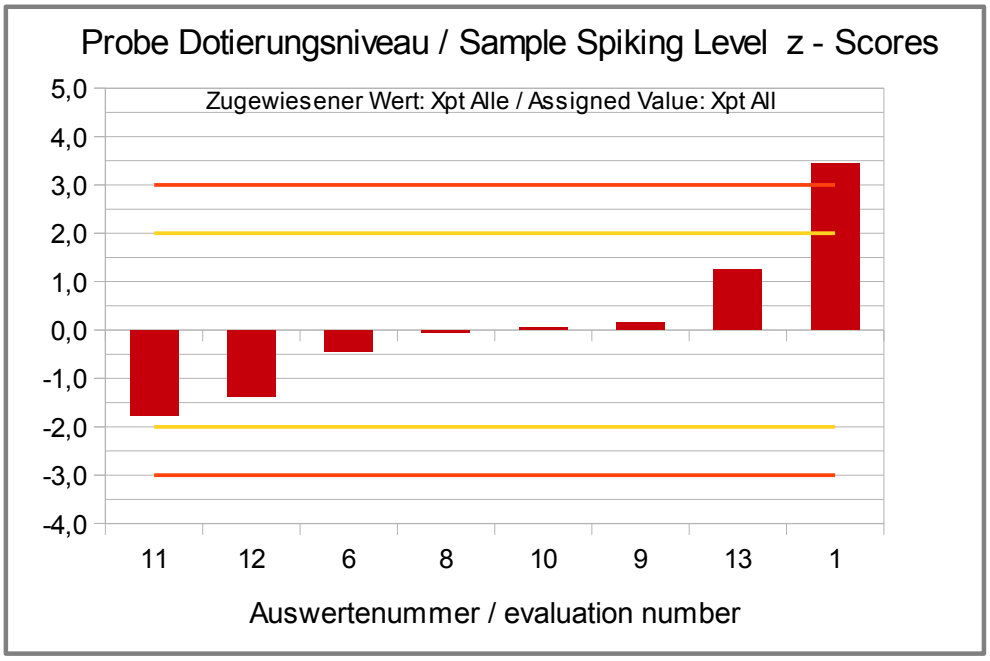


Abb./Fig. 7:
z-Scores (ELISA Results as milk protein) Assigned value robust mean of all results

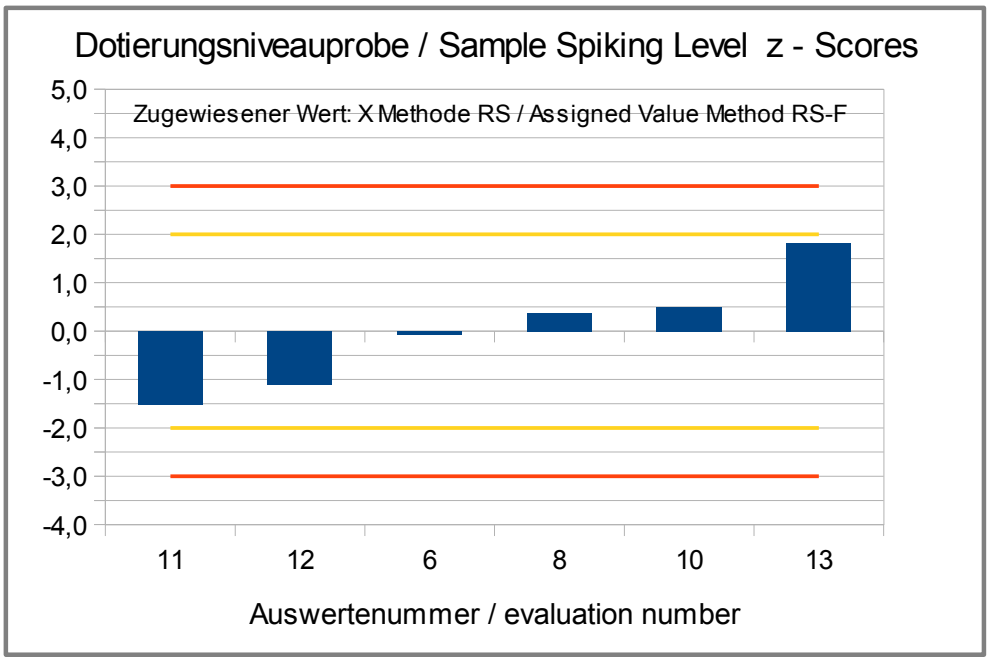


Abb./Fig. 8:
z-Scores (ELISA Results as milk protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates for Milk (as milk protein):
Spiking level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	52,4	**138**	1,79	4	AQ	Result converted °
6	25,0	**66**	14,9	36	RS-F	
8	27,8	**73**	6,99	17	RS-F	
10	28,6	**75**	7,20	17	RS-F	
11	15,7	41	18,8	45	RS-F	
12	18,4	49	10,3	25	RS-F	
13	37,0	**98**	7,33	18	RS-F	
9	29,3	**77**	2,56	6	VT	Result converted °

° Conversion p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	**6**	Anzahl im AB	**0**
Percent in RA	**75**	Prozent im AB	**0**

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

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
For the spiking level sample 75% (6) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B none of the participants obtained a recovery rate within the range of acceptance.

4.1.2 ELISA Results: Casein

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		
2	negative	<1	positive	1,60	2/2 (100%)	AQ	
3	negative		positive	1,37	2/2 (100%)	AQ	
7a	negative	<1	positive	1,70	2/2 (100%)	AQ	
8a	negative	< 0,2	positive	2,34	2/2 (100%)	IL	
7b	negative	<1	positive	11,0	2/2 (100%)	MI	
4	negative	<1,36	positive	3,90	2/2 (100%)	RS-F	
6	negative	<2,5	positive	9,07	2/2 (100%)	RS-F	
8b	negative	<2,5	positive	5,50	2/2 (100%)	RS-F	8b and 8c: different extractions
8c	negative	< 0,5	positive	1,48	2/2 (100%)	RS-F	8b and 8c: different extractions
9	negative		positive	7,91	2/2 (100%)	RS-F	
10	negative	<2,5	positive	7,20	2/2 (100%)	RS-F	
12	negative	< LOD	positive	4,74	2/2 (100%)	RS-F	
13	negative	<2.5	positive	5,66	2/2 (100%)	RS-F	

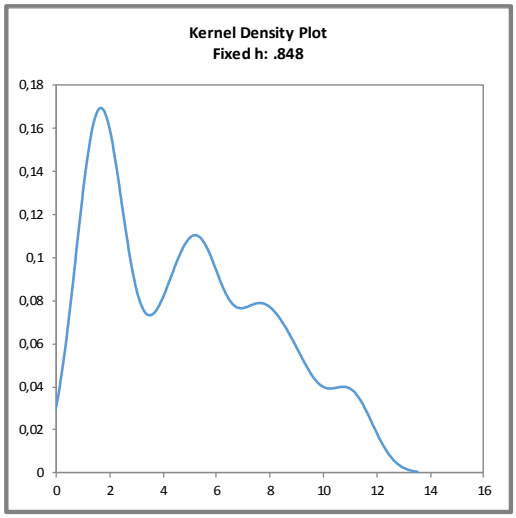
	Sample A		Sample B	
Number positive	0		13	
Number negative	13		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
AQ = AgraQuant, RomerLabs
IL = Immunolab
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm

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

Quantitative valuation of results: Sample B

Evaluation number	Casein	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	1,60	-1,9		AQ	
3	1,37	-2,0		AQ	
7a	1,70	-1,8		AQ	
8a	2,34	-1,5		IL	
7b	11,0	3,7		MI	
4	3,90	-0,5	-1,3	RS-F	
6	9,07	2,5	2,4	RS-F	
8b	5,50	0,4	-0,1	RS-F	8b and 8c: different extractions
8c	1,48	-2,0	-3,0	RS-F	8b and 8c: different extractions
9	7,91	1,8	1,5	RS-F	
10	7,20	1,4	1,0	RS-F	
12	4,74	0,0	-0,7	RS-F	
13	5,66	0,5	0,0	RS-F	



Methods:

AQ = AgraQuant, RomerLabs

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

Abb. / Fig. 9:

Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,5 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,5 x σpt of Xpt_{ALL})

Comments:

The kernel density estimation shows a main maximum at < 2,5 mg/kg, due to the results of three different methods (AQ, IL, RS-F). Further there are some side-peaks in decreasing intensity at > 4 mg/kg, due to results of method RS-F and a single value of method MI.

Characteristics: Quantitative evaluation Casein

Sample B

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	13	8
Number of outliers	0	0
Mean	4,88	5,68
Median	4,74	5,58
Robust Mean (X)	**4,80**	**5,71**
Robust standard deviation (S*)	**3,45**	**2,66**
Target range:		
Target standard deviation σ_{pt} and $\sigma_{pt'}$	**1,70**	**1,43**
lower limit of target range	**1,41**	**2,86**
upper limit of target range	**8,20**	**8,57**
Quotient S/σ_{pt}*	*2,0*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*1,20*	*1,18*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,7*	*0,8*
Results in the target range	*10*	*6*
Percent in the target range	*77*	*75*

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

Comments to the statistical characteristics and assigned values:

The kernel density plot showed no clear method dependent differences. The measured results were relatively low, with good agreement between median, arithmetic and robust mean.

The evaluation of results of all methods showed an increased variability of results. The quotient S/σ_{pt} was 2,9. Thus the evaluation was performed by z´-scores considering the standard uncertainty. The quotient S*/σ_{pt}` was then 2,0.*
The evaluation of the results from method RS-F 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 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 some methods.

The robust means of the evaluations were 14% and 17% of the spiking level of casein to sample B and thus below the recommendations for the applied methods (s. 3.4.3 and "recovery rates for casein", see page 39).

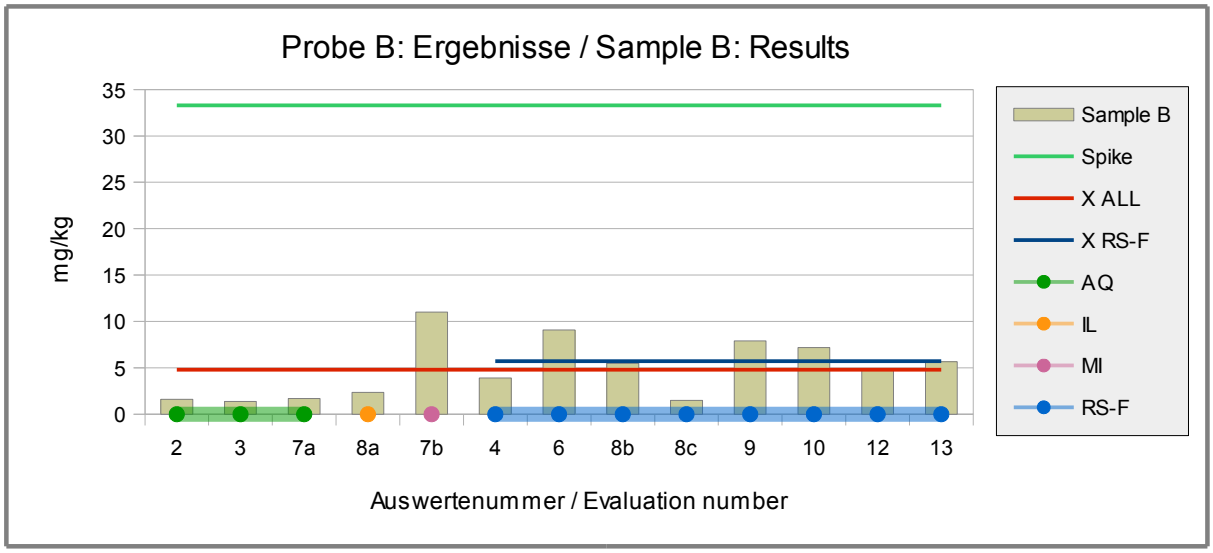


Abb./Fig. 10: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

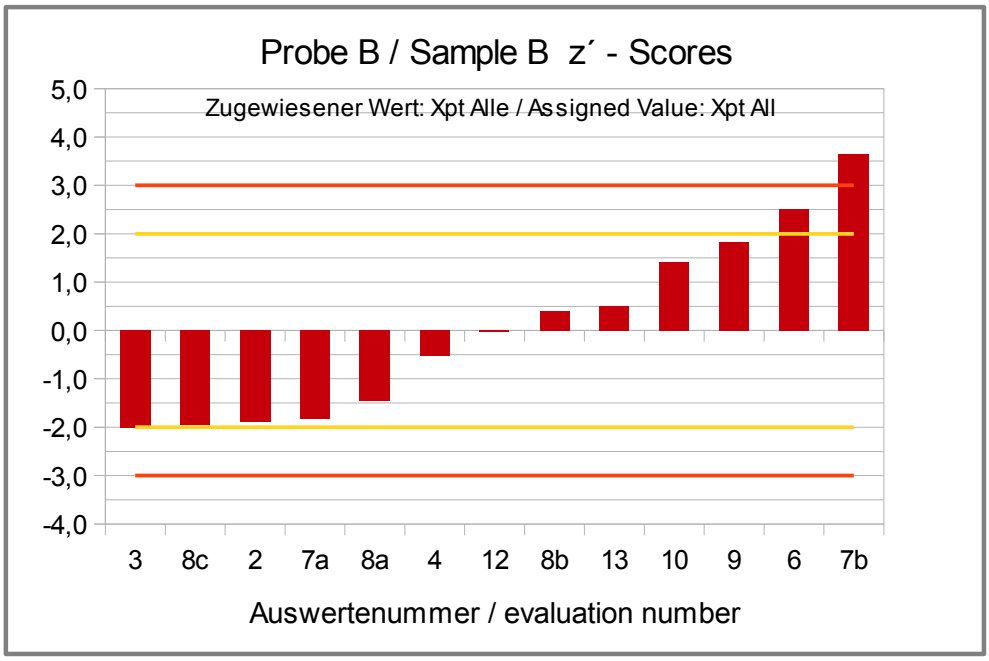


Abb./Fig. 11:
z´-Scores (ELISA Results Casein) Assigned value robust mean of all results

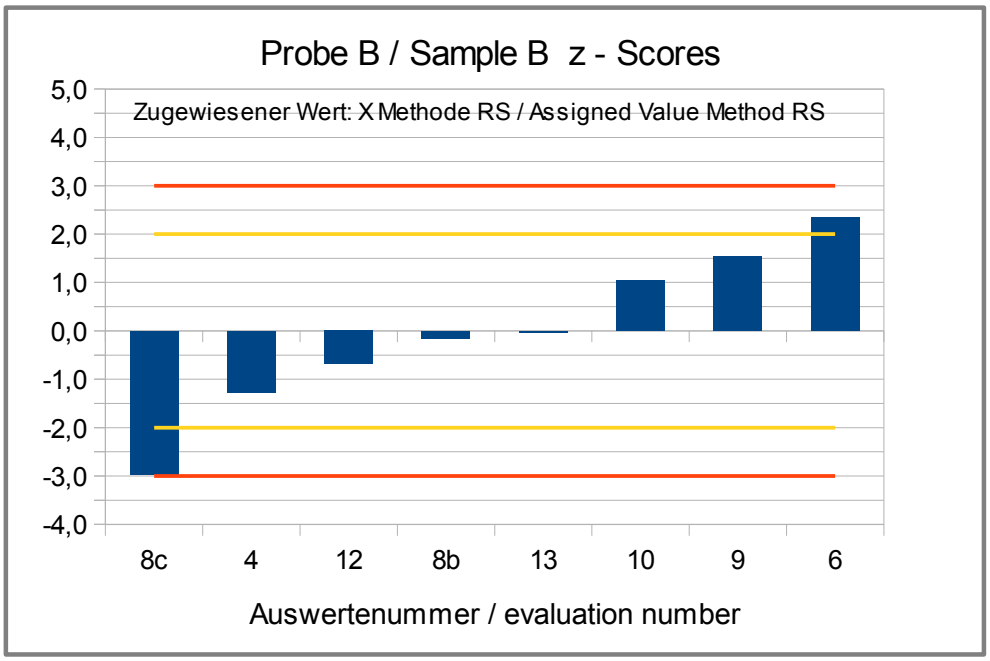


Abb./Fig. 12:
z-Scores (ELISA Results Casein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Casein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	32,8	0,5		AQ	
3	27,2	-0,3		AQ	
7	40,0	1,5		AQ	
8a	33,6	0,6		IL	
7	52,0	3,1		MI	
4	18,0	-1,5	-0,9	RS-F	
6	16,3	-1,8	-1,2	RS-F	
8b	29,4	0,0	1,0	RS-F	8b and 8c: different extractions
8c	17,4	-1,6	-1,0	RS-F	8b and 8c: different extractions
9	19,5	-1,3	-0,7	RS-F	
10	48,4	2,6	4,3	RS-F	
12	17,5	-1,6	-1,0	RS-F	
13	32,4	0,4	1,5	RS-F	

Methods:

AQ = AgraQuant, RomerLabs

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

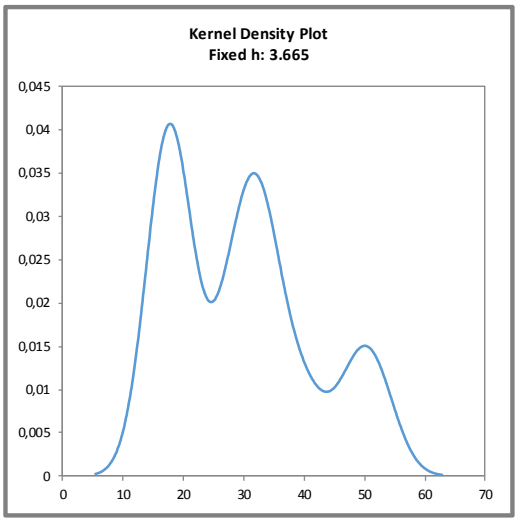


Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,5 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,5 x σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows a two main maximums at 15-20 mg/kg and 25-35 mg/kg and an additional side-peak > 30 mg/kg, which revealed no method dependent relation.

Characteristics: Quantitative evaluation Casein

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	13	8
Number of outliers	0	0
Mean	29,6	24,9
Median	29,4	18,7
Robust Mean (X)	**29,3**	**23,5**
Robust standard deviation (S*)	**13,0**	**9,33**
Target range:		
Target standard deviation σ_{pt}	**7,33**	**5,86**
lower limit of target range	**14,7**	**11,7**
upper limit of target range	**44,0**	**35,2**
Quotient S/σ_{pt}*	*1,8*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*4,51*	*4,12*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,6*	*0,7*
Results in the target range	*11*	*7*
Percent in the target range	*85*	*88*

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

Comments to the statistical characteristics and assigned values:

Both, the evaluation of the results of all methods and the results from method RS-F showed an 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 97% and 78% of the spiking level of casein to the spiking level sample and thus fitting the recommendations for the applied methods (s. 3.4.3 and "recovery rates for casein", see page 39).

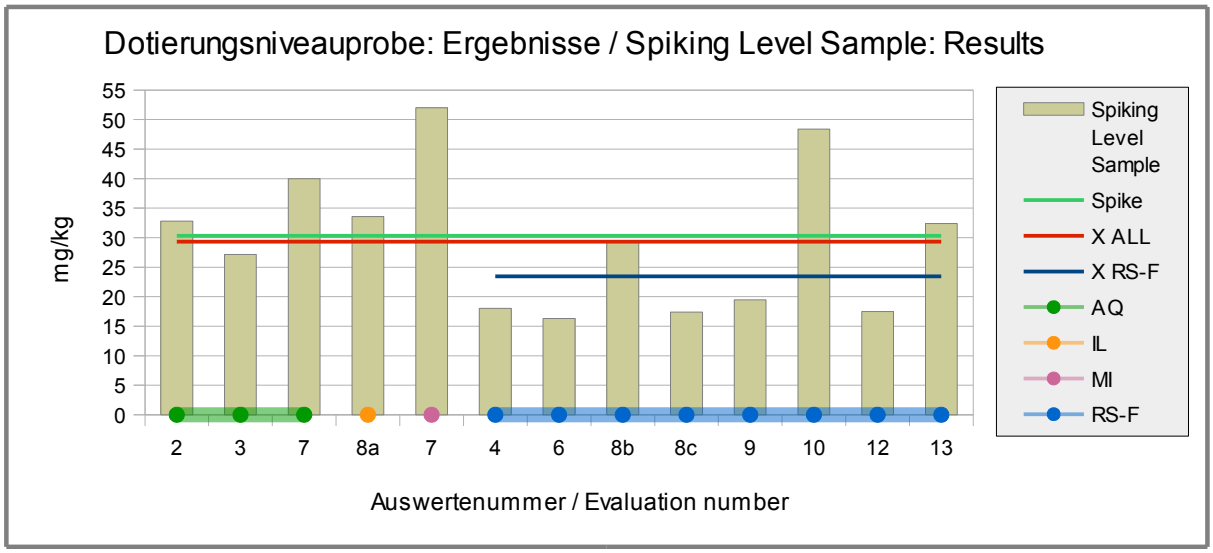


Abb./Fig. 14: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

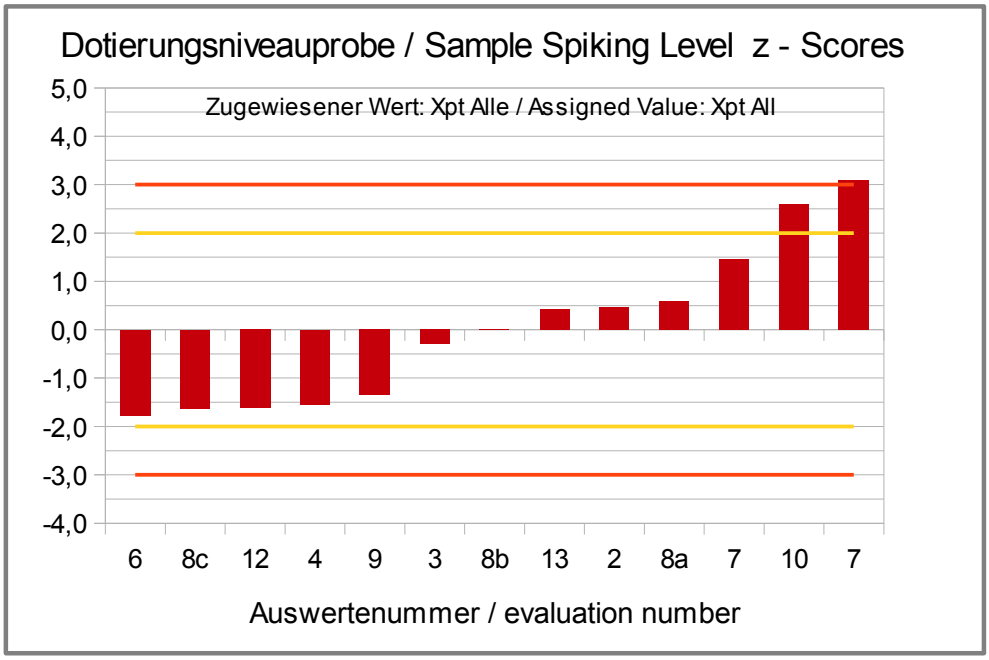


Abb./Fig. 15:
z-Scores (ELISA Results Casein) Assigned value robust mean of all results

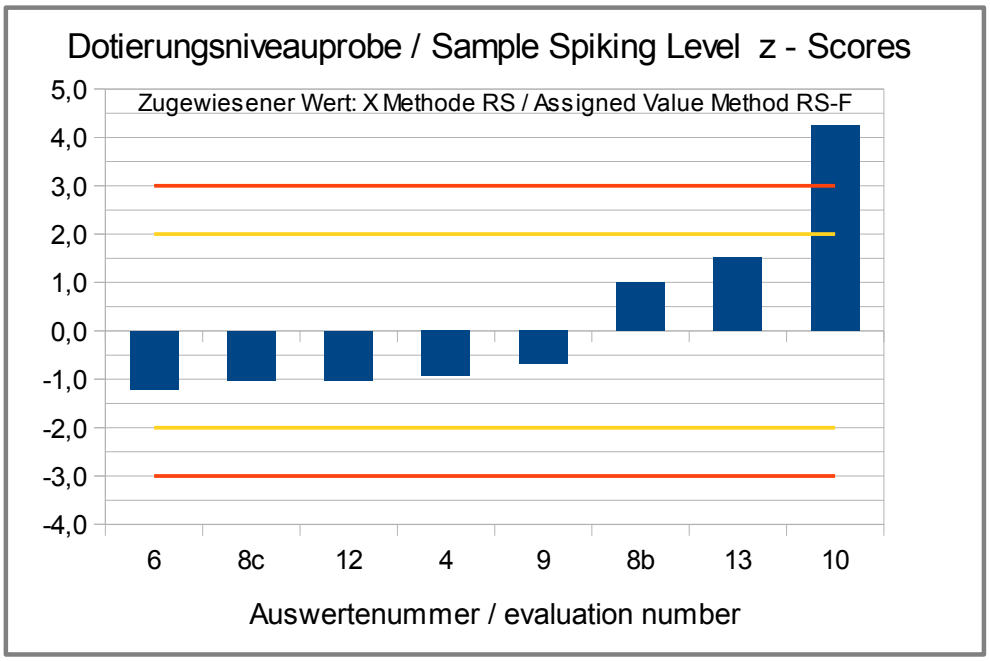


Abb./Fig. 16:
z-Scores (ELISA Results Casein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates for Casein:
Spiking level sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	32,8	**108**	1,60	5	AQ	
3	27,2	**90**	1,37	4	AQ	
7	40,0	**132**	1,70	5	AQ	
8a	33,6	**111**	2,34	7	IL	
7	52,0	172	11,0	33	MI	
4	18,0	**59**	3,90	12	RS-F	
6	16,3	**54**	9,07	27	RS-F	
8b	29,4	**97**	5,50	17	RS-F	8b and 8c: different extractions
8c	17,4	**57**	1,48	4	RS-F	8b and 8c: different extractions
9	19,5	**64**	7,91	24	RS-F	
10	48,4	160	7,20	22	RS-F	
12	17,5	**58**	4,74	14	RS-F	
13	32,4	**107**	5,66	17	RS-F	

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Anzahl im AB	**0**
Percent in RA	**85**	Prozent im AB	**0**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
AQ = AgraQuant, RomerLabs
IL = Immunolab
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
For the spiking level sample 85% (11) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B none of the obtained recovery rates were within the recommended range.

4.2 Proficiency Test Soya

4.2.1 ELISA Results: Soya (as soy protein)

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 *spiking of samples*		
2	negative	<10	negative	<10	1/2 (50%)	BC	
3	negative		negative		1/2 (50%)	BK	
4	negative	<2.5	negative	<2.5	1/2 (50%)	ES	
7	negative	<2,5	positive	52,0	2/2 (100%)	MI	
6	negative	<2,5	positive	47,3	2/2 (100%)	RS-F	
8	negative	< 2,5	positive	35,7	2/2 (100%)	RS-F	
9	negative		positive	49,7	2/2 (100%)	RS-F	
12	negative	<LOD	positive	26,6	2/2 (100%)	RS-F	
13	negative	<2.5	positive	36,0	2/2 (100%)	RS-F	
5	negative	<1,2	negative	<1,2	1/2 (50%)	VT	Result converted °
13	negative	<1,2	negative	<1,2	1/2 (50%)	VT	Result converted °

° Conversion p. 18

	Sample A		Sample B	
Number positive	0		6	
Number negative	11		5	
Percent positive	0		55	
Percent negative	100		45	
Consensus value	negative		none	

Methods:

BC = BioCheck ELISA
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

** agreement with spiking of samples*

Comments:

The results for sample A are in qualitative agreement with the spiking of sample B. For sample B there was no consensus value with ≥75% positive or negative results. The methods MI and RS-F provided consistent positive results in agreement with the spiking of sample B, while the other methods provided exclusively negative results. The qualitative valuation of results was therefore carried out by comparing them with the spiking of the samples.

Quantitative valuation of results: Sample B

Evaluation number	Soy protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Methode	Hinweis
	[mg/kg]				
2	<10			BC	
3				BK	
4	<2.5			ES	
7	52,0	1,0		MI	
6	47,3	0,6	0,8	RS-F	
8	35,7	-0,5	-0,3	RS-F	
9	49,7	0,8	1,1	RS-F	
12	26,6	-1,4	-1,3	RS-F	
13	36,0	-0,5	-0,3	RS-F	
5	<1,2			VT	Result converted °
13	<1,2			VT	Result converted °

° Conversion p. 18

Methods:

BC = BioCheck ELISA
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
Due to the low number < 8 of results the kernel density was not evaluated.

Characteristics: Quantitative evaluation Soya

Sample B

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	6	5
Number of outliers	0	0
Mean	41,2	39,1
Median	41,7	36,0
Robust Mean (X)	**41,2**	**39,1**
Robust standard deviation (S*)	**11,3**	**10,7**
Target range:		
Target standard deviation σ_{pt}	**10,3**	**9,77**
lower limit of target range	**20,6**	**19,5**
upper limit of target range	**61,8**	**58,6**
Quotient S/σ_{pt}*	*1,1*	*1,1*
Standard uncertainty $U_{(Xpt)}$	*5,78*	*6,00*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,60*	*0,60*
Results in the target range	*6*	*5*
Percent in the target range	*100*	*100*

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

Comments to the statistical characteristics and assigned values:

The evaluation of results of all methods and of results of method RS-F showed a normal to low variability of results. The quotients S/σ_{pt} were 1,1 each.*
The robust standard deviation is in the range of established values for the repeatablility 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.

All results were in the target range.

The robust means of the evaluations were 49% and 47% of the spiking level of soya protein to sample B and thus slightly below the recommendations for the applied methods (s. 3.4.3 and "recovery rates for soya", see page 39).

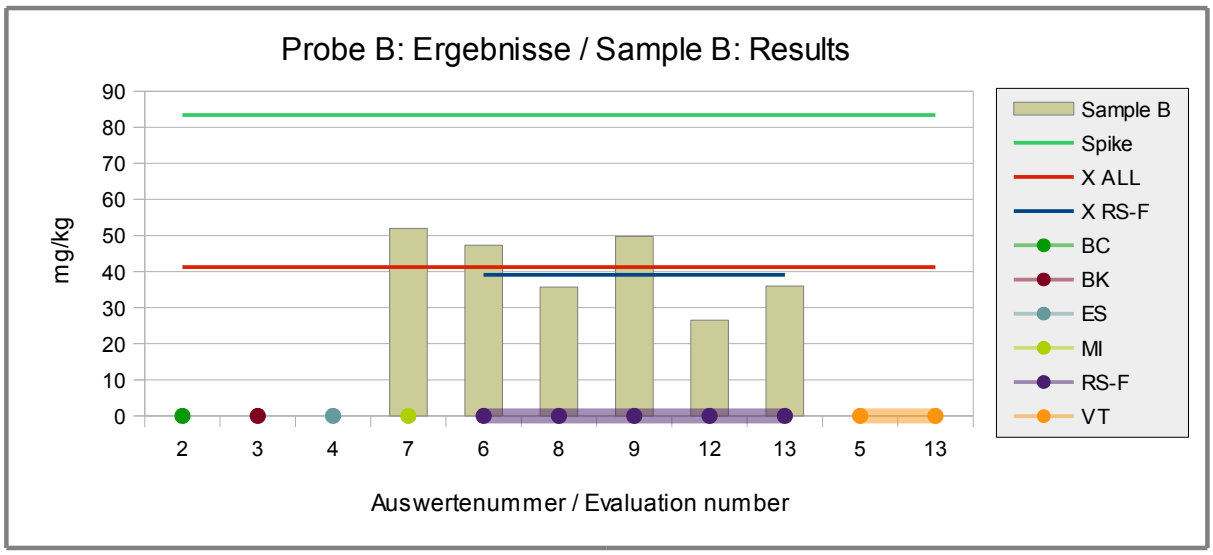


Abb./Fig. 17: ELISA Results Soya (as soy protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

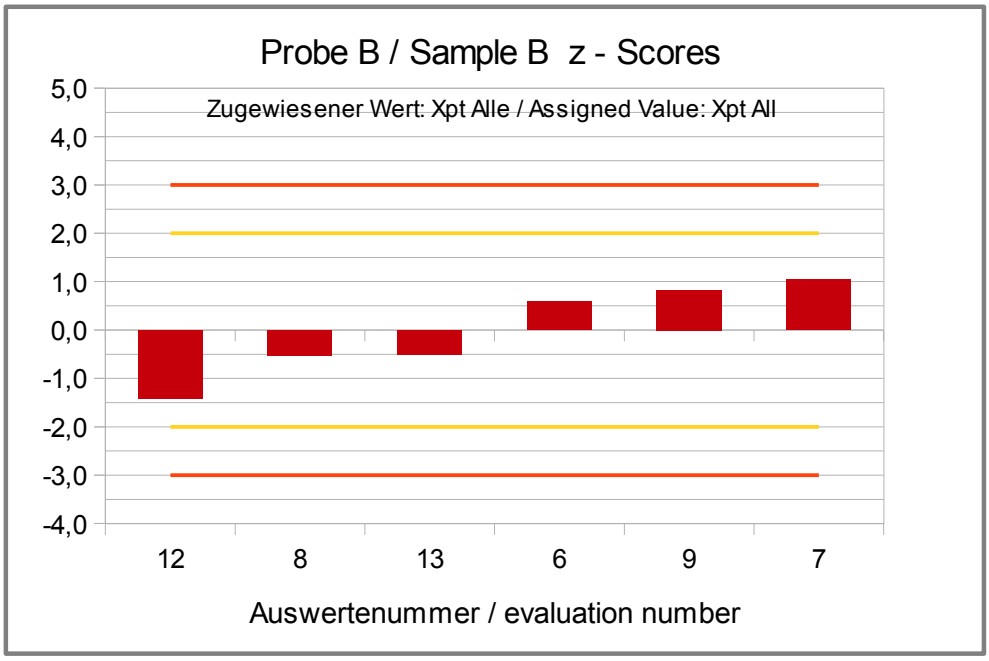


Abb./Fig. 18:
z-Scores (ELISA Results as soya protein) Assigned value robust mean of all results

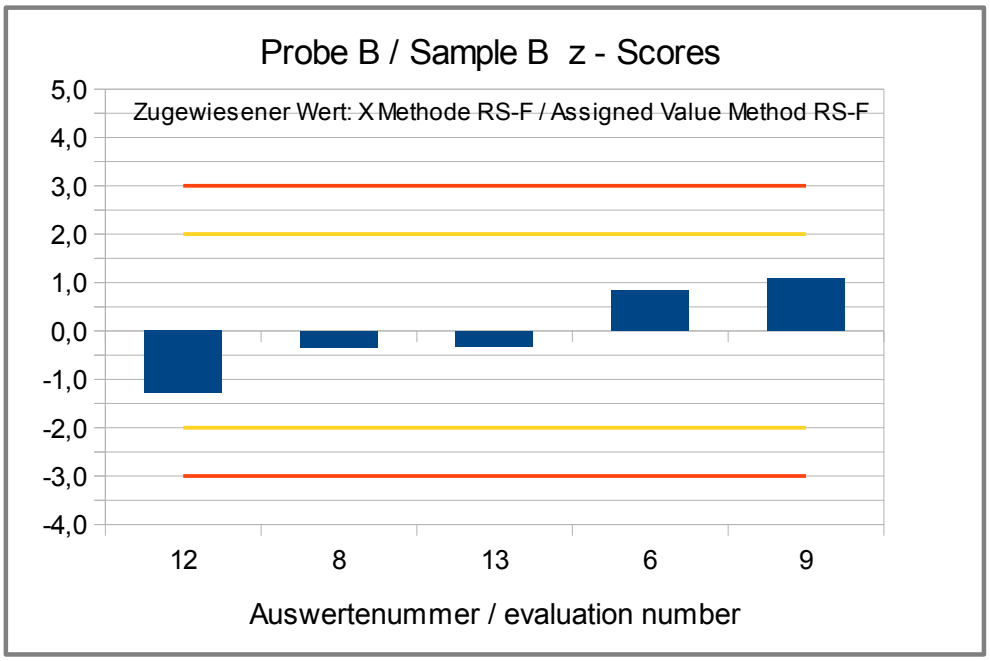


Abb./Fig. 19:
z-Scores (ELISA Results as soya protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Soy protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Methode	Hinweis
	[mg/kg]				
2	265	18,7		BC	Result excluded
3				BK	
4	86,0	3,4		ES	
7	260	18,3		MI	Result excluded
6	33,6	-1,1	-1,0	RS-F	
8	54,1	0,6	0,9	RS-F	
9	43,8	-0,2	0,0	RS-F	
12	29,3	-1,5	-1,4	RS-F	
13	60,4	1,2	1,5	RS-F	
5	35,7	-0,9		VT	Result converted °
13	45,6	-0,1		VT	Result converted °

° Conversion p. 18

Methods:

BC = BioCheck ELISA
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

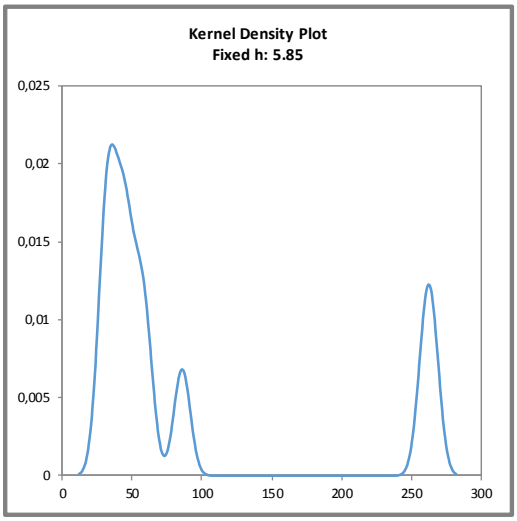


Abb. / Fig. 20:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,5 x σ_{pt} von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,5 x σ_{pt} of Xpt_{ALL})

Comments:
The kernel density estimation shows a main maximum with a side peak at <100 mg/kg and a further side peak at >250 mg/kg, due to the two excluded results.

Characteristics: Quantitative evaluation Soya (as soy protein)

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	8	5
Number of outliers	0	0
Mean	48,6	44,2
Median	44,7	43,8
Robust Mean (X)	**46,7**	**44,2**
Robust standard deviation (S*)	**16,3**	**14,9**
Target range:		
Target standard deviation σ_{pt} and $\sigma_{pt'}$	**11,7**	**11,1**
lower limit of target range	**23,3**	**22,1**
upper limit of target range	**70,0**	**66,3**
Quotient S/σ_{pt}*	*1,4*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*7,19*	*8,35*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,60*	*0,80*
Results in the target range	*7*	*5*
Percent in the target range	*88*	*100*

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

Comments to the statistical characteristics and assigned values:
The kernel density estimation showed nearly a normal distribution of results except for the both excluded results and a single value out-side the target range.

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, be-cause there were only a few results for the methods.

88% to 100% of results were in the target range.

The robust means of the evaluations were 125% and 118% of the spiking level of soy protein to the spiking level sample and thus fitting the recommendations for the applied methods (s. 3.4.3 and "recovery rates for soya", see page 49).

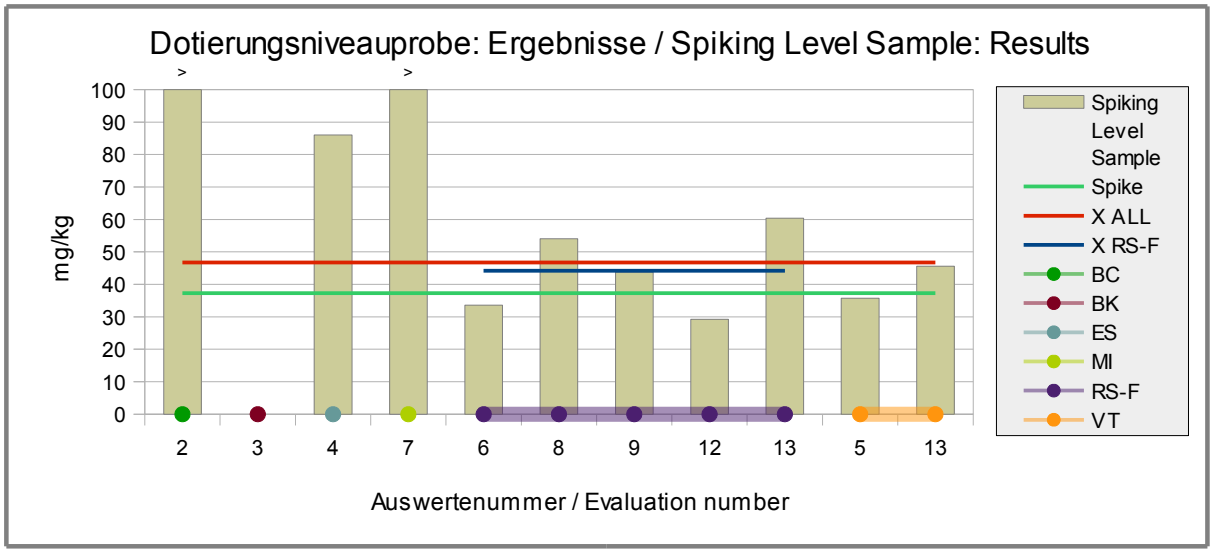


Abb./Fig. 21: ELISA Results Soya (as soy protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

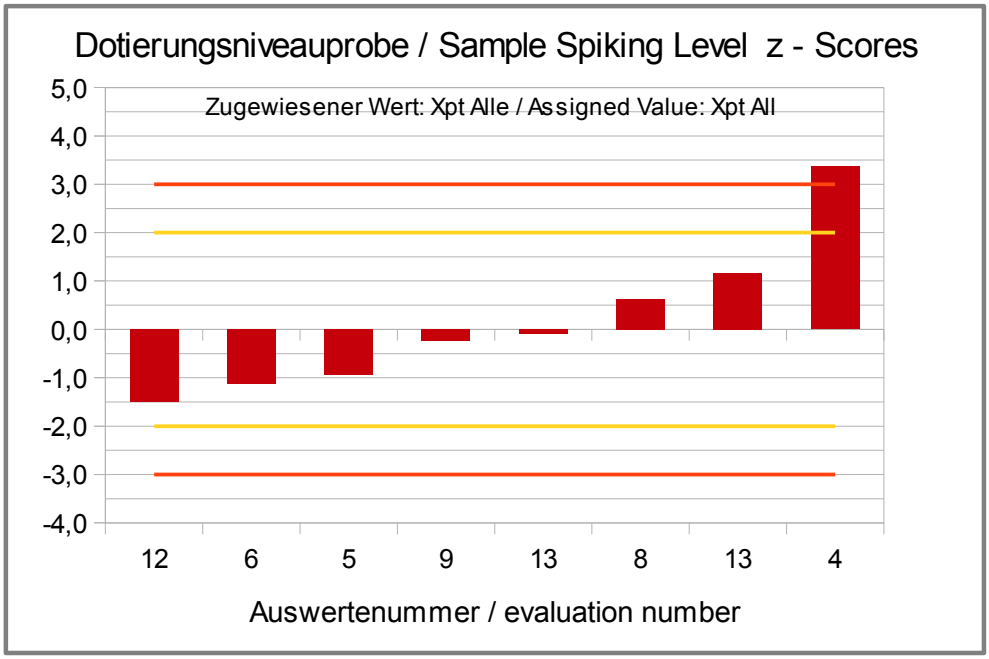


Abb./Fig. 22:
z-Scores (ELISA Results as soy protein) Assigned value robust mean of all results

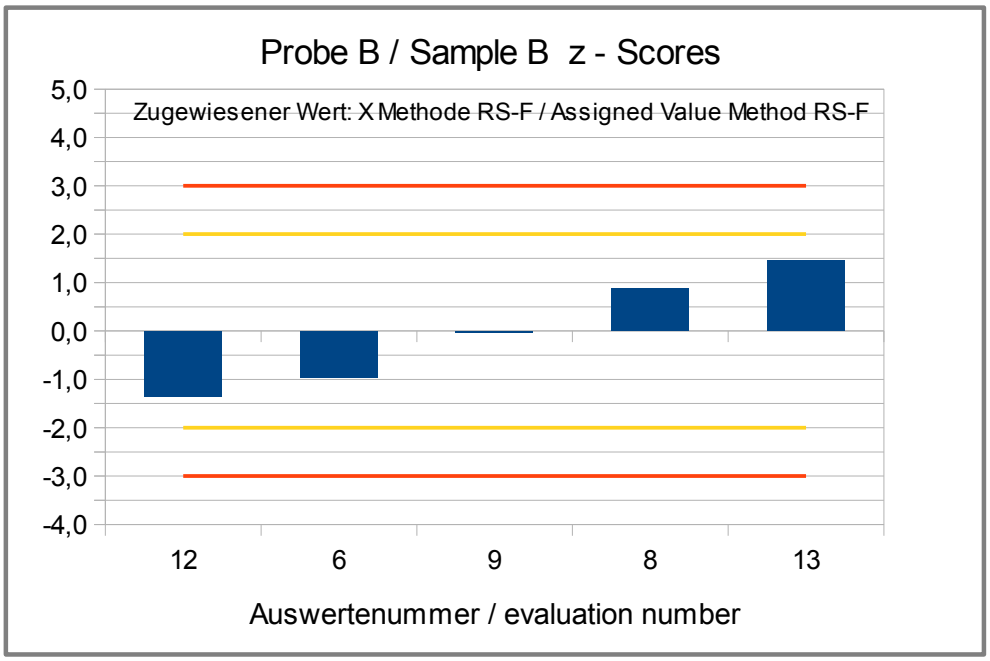


Abb./Fig. 23:
z-Scores (ELISA Results as soy protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates for Soya (as soya protein):
Spiking level sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	265	712	<10		BC	
3					BK	
4	86,0	231	<2,5		ES	
7	260	697	52,0	**62**	MI	
6	33,6	**90**	47,3	**57**	RS-F	
8	54,1	**145**	35,7	43	RS-F	
9	43,8	**117**	49,7	**60**	RS-F	
12	29,3	**78**	26,6	32	RS-F	
13a	60,4	162	36,0	43	RS-F	
5	35,7	**96**	<1,2		VT	Result converted °
13b	45,6	**122**	<1,2		VT	Result converted °

° Conversion p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	6	Anzahl im AB	3
Percent in RA	60	Prozent im AB	50

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

** Akzeptanzbereich der AOAC für Allergen-ELISAs

Methods:
BC = BioCheck ELISA
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking level sample 60% (6) of the participants obtained a recovery rate by ELISA within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B 50% (3) of the obtained recovery rates were within and the other 50% slightly below the recommended range.

4.2.2 PCR Results: Soya (as Soybean / Soy flour)

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		
3	negative		positive		2/2 (100%)	ASU	
6	negative		positive		2/2 (100%)	ASU	
9	negative		positive		2/2 (100%)	ASU	
13	negative	<1	positive	44,2	2/2 (100%)	SFA-ID	
7	negative		positive		2/2 (100%)	div.	

	Sample A		Sample B	
Number positive	0		5	
Number negative	5		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

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

Quantitative valuation of results: Sample B

There were only a single quantitative result, therefore no statistical evaluation was done.

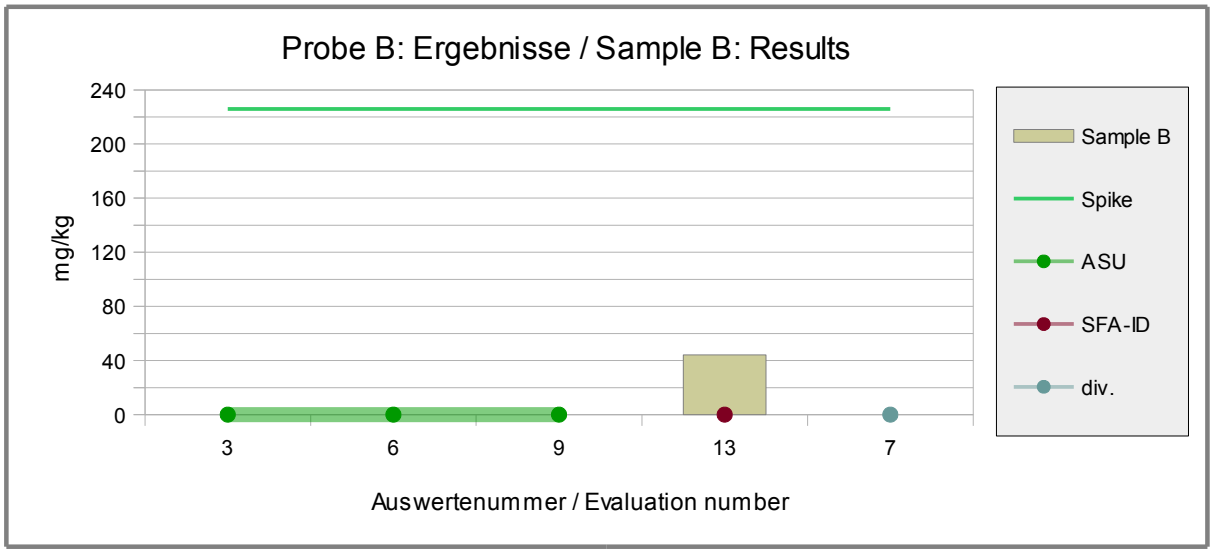


Abb./Fig. 24: PCR-Results Soya (as soybean / soy flour)
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Quantitative valuation of results: Spiking level sample

There were only a single quantitative result, therefore no statistical evaluation was done.

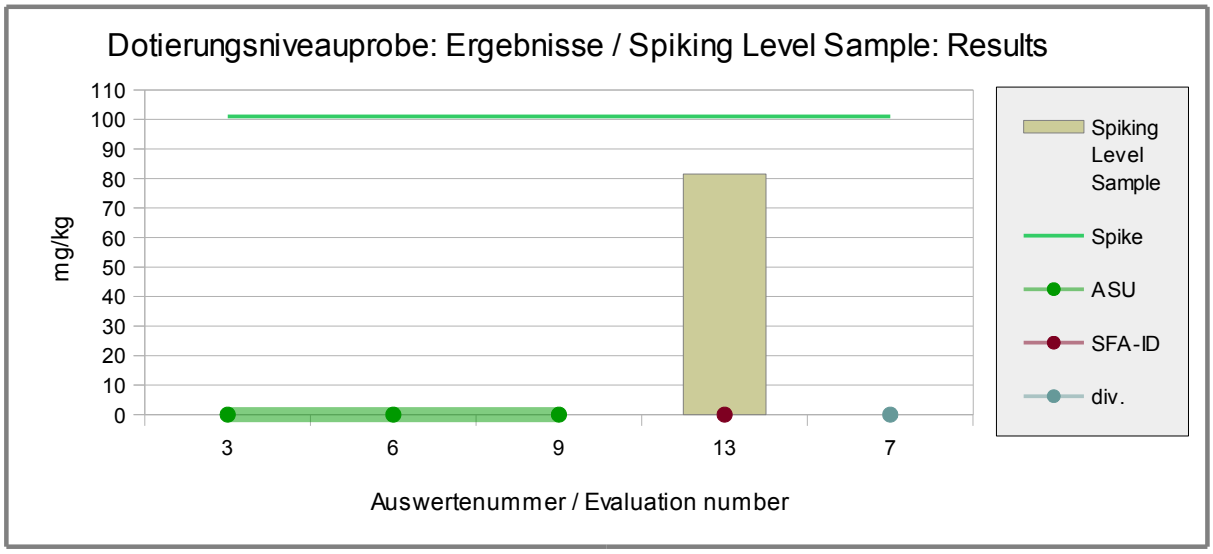


Abb./Fig. 25: PCR-Results Soya (as soya protein)
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Recovery Rates for Soya (as soya protein):
Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3					ASU	
6					ASU	
9					ASU	
13	81,49	**81**	44,2	20	SFA-ID	
7					div.	

RA*	50-150 %	RA*	50-150 %
Number in RA	1	Anzahl im AB	0
Percent in RA	100	Prozent im AB	0

* Recovery rate 100% relative size: soya, s. page 4

** Range of acceptance of AOAC for allergen ELISAS

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

Comments:
One participant submitted a quantitative result and obtained a recovery rate for the spiking level sample within the range of the AOAC-recommendation of 50-150%. For the processed and spiked food matrix sample B the obtained recovery rate was 20% and thus below 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: Milk

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	27.02.17	-	0,484	-	6,644	-	194,04	food	AgraQuant ELISA Milk COKAL2448, RomerLabs
RS-F	6	13.02.17	negative	<2,5	positive	14,9	positive	24,98	Milk protein, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	8	20./21.01.	negative	< 2,5	positive	6,99	positive	27,78	Milk protein, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	10	14.02.17	negative	<2,5	positive	7,2	positive	28,6	protein	r-biopharm, RIDASCREEN®FAST Milk (R4652)
RS-F	11	09.02.17	negative	<2,5	positive	18,84	positive	15,7	Please choose!	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	12	25.01.17	-	< LOD	-	10,32	-	18,42	Milk protein, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	13	27.01.17	negative	<2.5	positive	7,33	positive	36,96	Milk protein, total	Ridascreen® FAST Milk R4652, R-Biopharm
VT	9	10.02.17	negative		positive	7,3	positive	83,42	skimmed milk powder	Veratox Total Milk Allergen, Neogen

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	1		If whole milk powder => 194,04 mg/kg Spiking Sample; A 0,484 mg/kg; B= 6,644 mg/kg.	Spiking Sample=Present with ETMILK-1004
RS-F	6		As Per Kit Instructions	Sample material seems to be inhomogenous after arrival. During heating process meat juice is emitted. A rough homogenization was perfomred using a mortar (sample quantity is to low)
RS-F	8		As Per Kit Instructions	
RS-F	10		As Per Kit Instructions	
RS-F	11	see kit instruction	Processing of samples exactly as per kit instructions	Quantitative result as mean of three measurements
RS-F	12	Milk protein, total		
RS-F	13	As Per Kit Instructions	As Per Kit Instructions	
VT	9			Sample quantity was to low for a double determination

5.1.2 ELISA: Casein

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	27.02.17	-	0,11	-	1,51	-	44,1	food	AgraQuant ELISA Milk COKAL2448, RomerLabs
AQ	2	03.03.17	negative	<1	positive	1,6	positive	32,8	Please choose!	Romer Casein Kit
AQ	3	03.03.17	negative		positive	1,37	positive	27,15	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	7a	20.01.	negative	<1	positive	1,7	positive	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
IL	8a	21.02.	negative	< 0,2	positive	2,34	positive	33,56	Casein	Immunolab Casein ELISA
Mi	7b	20.01.	negative	<1	positive	11	positive	52	Casein	Morinaga Casein ELISA Kit
RS-F	4	02.03.17	negative	<1.36	positive	3,9	positive	18	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	6	02.03.17	negative	<2,5	positive	9,07	positive	16,31	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	8b	21.02.	negative	< 2,5	positive	5,5	positive	29,35	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	8c	21.02.	negative	< 0,5	positive	1,48	positive	17,39	Casein	RIDASCREEN® FAST Casein R4612, R-Biopharm
RS-F	9	08.02.17	negative		positive	7,91	positive	19,46	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	10	13.02.17	negative	<2,5	positive	7,2	positive	48,4	Casein	r-biopharm, RIDASCREEN®FAST Casein (R4612)
RS-F	12	23.01.17	-	< LOD	-	4,74	-	17,48	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	13	23.01.17	negative	<2.5	positive	5,66	positive	32,41	Casein	Ridascreen® FAST Casein R4612, R-Biopharm

Continuation ELISA: Casein

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	1			Spiking Sample=Present w ith ETMILK-1004
AQ	2	Casein	As Per Kit Instructions	
AQ	3	Casein	nach Testkitanleitung	NG = 0,2 mg/kg
AQ	7a	Casein	As Per Kit Instructions	
IL	8a		As Per Kit Instructions	
Mi	7b		As Per Kit Instructions	Kit II
RS-F	4			
RS-F	6		As Per Kit Instructions	s. Milk
RS-F	8b		As Per Kit Instructions w ith Extractor 2	
RS-F	8c		As Per Kit Instructions w ith Extractor 2	
RS-F	9		Extraction mit Extractor 2	Sample quantity w as to low for a double determination
RS-F	10		As Per Kit Instructions	
RS-F	12	Casein		
RS-F	13	As Per Kit Instructions	As Per Kit Instructions	

5.1.3 ELISA: Soya

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
BC	2	03.03.17	negative	<10	negative	<10	positive	265,4	Roasted soya protein	Biocheck - Soya Check
BK	3	16.02.17	negative		negative		negative		Soyprotein	Neogen Biokits 902001T
ES	4	02.03.17	negative	<2.5	negative	<2.5	positive	86	Soyprotein	ELISA Systems Soy ESSOYPRD-48
Mi	7	24.02.	negative	<2,5	positive	52	positive	260	Soyprotein	Morinaga Soya ELISA Kit II
RS-F	6	10.02.17	negative	<2,5	positive	47,3	positive	33,61	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	8	27.01./21.02.	negative	< 2,5	positive	35,74	positive	54,05	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	9	23.01.17	negative		positive	49,74	positive	43,82	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	12	23.01.17	-	<LOD	-	26,56	-	29,27	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	13	10.02.17	negative	<2.5	positive	36	positive	60,4	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
VT	5	02.03.17	-	<2.5	-	<2.5	-	76	PPM soy flour	Veratox for soy allergen kit 8410
VT	13	23.01.17	negative	<2.5	negative	<2.5	positive	97	Soyflour	Veratox Soy Allergen, Neogen

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
BC	2	Roasted soya protein	Method detects soya trypsin inhibitor - reported value is a conversion to roasted soya protein equivalent.	
BK	3	Soyprotein	As test kit instruction for a determinaton range of 0,7 - 14% Soyprotein; sample w eight = 12g; Processing B for low er concentration range not possible, due to the low sample quantity	Due to the positive PCR result gained fpr sample B the concentration must be < 0,7% Soyprotein. Further the spiking level sample must exhibit a concentration of <0,7% Sojprotein, as it w as not verifiable via ELISA in the range of 0,7 – 14%
ES	4			
Mi	7		As Per Kit Instructions	
RS-F	6		As Per Kit Instructions	s. Milk
RS-F	8		As Per Kit Instructions	
RS-F	9			
RS-F	12	Soyprotein		
RS-F	13	As Per Kit Instructions	As Per Kit Instructions	
VT	5			
VT	13	As Per Kit Instructions	As Per Kit Instructions	

5.1.4 PCR: Soya

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	3	16.02.17	negative		positive		-		Soya-DNA	ASU §64 Methode/method
ASU	6		negative		positive		positive		Soya-DNA	ASU § 64 LFGB L 00.00-105, Anhang C.2 (modifiziert)
ASU	9	30.01.17	negative		positive		positive		Soya-specific DNA Sequences	§64 LFGB L 08-00-59
SFA-ID	13	26.01.17	negative	<1	positive	44,18	positive	81,49	Soybean	Sure Food Allergen ID, R-Biopharm / Congen
div.	7	20.01.	negative		positive		positive		Soya-DNA	other: please choose!

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Target Sequence / DNA	ction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	3	Lectin	Extraktion: NucleospinFood; Real-Time-PCR L08.00-59	
ASU	6	Lectin Gen (74 bp)	buffer with Proteinase K, Preocessing with Wizard-Kit der Fa. Promega)	Sample B: < 50 haploidentical genome copies; Spiking level sample: < 475 haploidentical genome copies
ASU	9	Lectin	Machery & Nagel NucleoSpin Food Kit	
SFA-ID	13	As Per Kit Instructions	As Per Kit Instructions	
div.	7		CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real Time PCR/45 Cyclen	Eur F Res Tech 216 (2003) 412ff, mod. (45 Cyclen)

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 01-2017 Spiking level sample

Weight whole sample	1,53	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,16	82	31,8
2	4,99	109	43,7
3	5,13	81	31,6
4	5,00	100	40,0
5	5,07	107	42,2
6	5,03	98	39,0
7	5,12	88	34,4
8	5,02	94	37,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	95,0	Partikel
Standard deviation	11,6	Partikel
χ^2 (CHI-Quadrat)	9,88	
Probability	**20**	%
Recovery rate	132	%

Normal distribution		
Number of samples	8	
Mean	37,5	mg/kg
Standard deviation	4,57	mg/kg
rel. Standard deviaton	12,2	%
Horwitz standard deviation	9,3	%
HorRat-value	**1,3**	
Recovery rate	132	%

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 01-2017***
PT name	***DLA 01/2017 - Allergens I: Milk (Casein) and Soya in Sausage with „Spiking Level Sample“***
Sample matrix	***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:* *Milk (milk protein, casein, DNA), Soya (Soyprotein, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. 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 March 3rd 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
		GREAT BRITAIN
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		ITALY
		ISRAEL
		Germany
		Germany
		GREAT BRITAIN
		Germany
		ITALY

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

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

7. Index of references

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

20. Ministry of Health and Welfare, JSM, Japan 2006
21. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
22. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
23. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
24. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
25. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
26. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
27. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003)
28. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006)
29. ASU §64 LFGB L 16.01-9 Untersuchung von Lebenmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016)
30. ASU §64 LFGB L 08.00-59 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013)
31. ASU §64 LFGB L 08.00-65 Untersuchung von Lebenmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba). Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2016)
32. Allergen Data Collection - Update (2002): Cow's Milk (Bos domesticus), Besler M., Eigenmann P., Schwartz R., Internet Symposium on Food Allergens 4(1): 19-106, http://www.food-allergens.de