Evaluation Report

proficiency test

DLA 01/2019

Allergens I:

Milk (Casein) and Soya

in Sauce Powder

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1st Correction 17/06/2019:

In table "Quantitative Evaluation ELISA: Sample A" for milk protein on page 37 was a mistake:
The z-scores for the evaluation numbers 12b, 6 and 17 were wrong. This has been corrected. The z-scores in fig. 15 were not affected (p. 40).

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

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EP-Nummer *PT-Number*	DLA 01/2019
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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (17 June 2019) 1. Korrektur / 1st Correction Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 17 June 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a customary instant sauce powder. The basic composition of samples A and B was the same (see table 1). After crushing and sieving (mesh 2,0 mm) the basic mixture was homogenized.
Afterwards the **spiked sample A** was produced as follows:
The spiking materials containing the allergenic ingredients skimmed milk powder and soyflour were crushed and sieved by a centrifugal mill (mesh 250 µm), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 4 additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh 500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Sauce powder Ingredients: Maltodextrin, starch, modified starch, iodised salt, wheat flour, sugar, flavorings, yeast extract, onions, spices, caramel, tomato powder, corn oil, malt extract, acidity regulators: sodium diacetate, calcium lactate, acidifier: citric acid, lactic acid, thyme Nutrients per 100 g: Protein <5,0 g, Carbohydrates 73 g, Fat <5,0 g, Salt 12 g	99,9 g/100 g	100 g/100g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,9 g/100 g
Milk skimmed milk powder mixture (9 products from Europe, USA) - as skimmed milk powder* - thereof 33,0% total protein** - thereof Casein*** - thereof β-Lactoglobulin***	 50,6 mg/kg 16,7 mg/kg 13,4 mg/kg 5,6 mg/kg # s.remarks	- (positive)	 53,3 mg/kg 17,6 mg/kg 14,1 mg/kg 5,3 mg/kg
Soya: soyflour-mixture, toasted (6 products from Asia, Europe, North Amerika) - as Soyflour* - *thereof 37,8 total protein*** - *thereof soy trypsin inhibitor****	 98,0 mg/kg 37,1 mg/kg 5,57 mg/kg	-	 98,0 mg/kg 37,1 mg/kg 5,57 mg/kg
further Ingredients: *Maltodextrin*	<0,1 g/100 g	-	<0,1 g/100 g

* Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=6,38 for milk protein and with F=5,71 for soyprotein)
*** Protein contents calculated according to literature values (approx. 80% casein and 10% β-latcoglobulin in total milk protein [36]; approx. 15% soy trypsin inhibitor in soy protein [37])

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

Sample A additionally contains total milk protein or casein from the matrix instant sauce powder, as determined in sample B. For the calculation of the recovery rates of the participants' results, therefore, the average contents of total milk protein and casein from sample B were taken into account. The following target values for sample A are obtained: ***total milk protein 44,0 mg/kg and Casein 34,4 mg/kg.***

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 A and the spiking level sample showed a probability of 86% and 74%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17].
This gave a HorRat value of 1,0 and 1,0 respectively. Aufgrund der ausreichenden Wahrscheinlichkeit wurde der HorRat-Wert für Probe B akzeptiert. The HorRat value of sample B was accepted because of the sufficient probability. 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 13528:2015 Annex B (possibly with Notes 1 and 2).

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

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

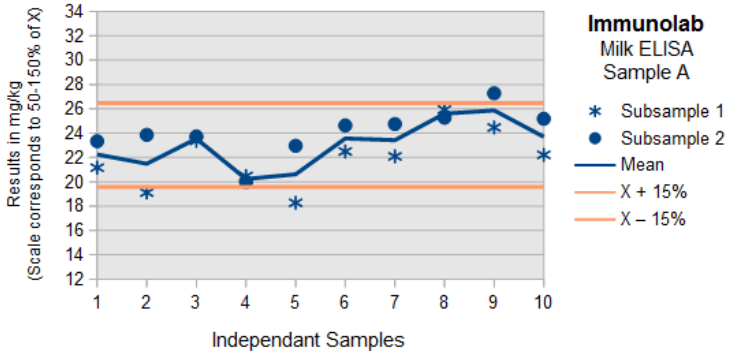
ELISA-Tests: Homogenität Milch / Homogeneity Milk

Immunolab Milk ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Sum of Casein + beta-Lactoglobulin 23,0 ± 1,6 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	21,2	23,3	22,2
2	19,1	23,9	21,5
3	23,4	23,7	23,5
4	20,5	20,0	20,2
5	18,3	23,0	20,6
6	22,5	24,6	23,6
7	22,1	24,7	23,4
8	25,9	25,3	25,6
9	24,5	27,3	25,9
10	22,2	25,2	23,7

General average X	23,0	
SD of sample means Sx	1,90	8,2%
SD within-samples Sw	1,40	6,1%
SD between-samples Ss	1,62	7,0%

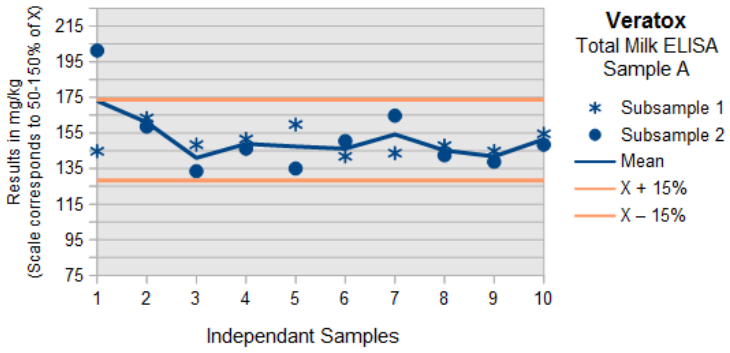


Neogen Veratox ELISA Total Milk

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Non fat dry milk 151 ± 6 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	145	201	173
2	163	159	161
3	148	134	141
4	152	146	149
5	160	135	147
6	142	150	146
7	144	165	154
8	148	142	145
9	145	139	142
10	155	148	151

General average X	151	
SD of sample means Sx	9,74	6,4%
SD within-samples Sw	10,8	7,2%
SD between-samples Ss	6,00	4,0%

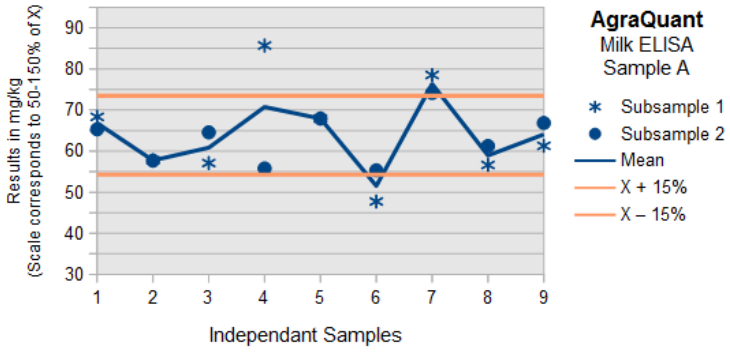


Romerlabs AgraQuant Milk

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Non fat dry milk 63,9 ± 6,4 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	68,3	65,3	66,8
2	57,8	57,7	57,7
3	57,1	64,6	60,8
4	85,7	55,8	70,7
5	67,9	67,9	67,9
6	47,7	55,3	51,5
7	78,5	74,1	76,3
8	56,6	61,2	58,9
9	61,3	66,8	64,1

General average X	63,9	
SD of sample means Sx	7,51	11,8%
SD within-samples Sw	5,50	8,6%
SD between-samples Ss	6,43	10,1%



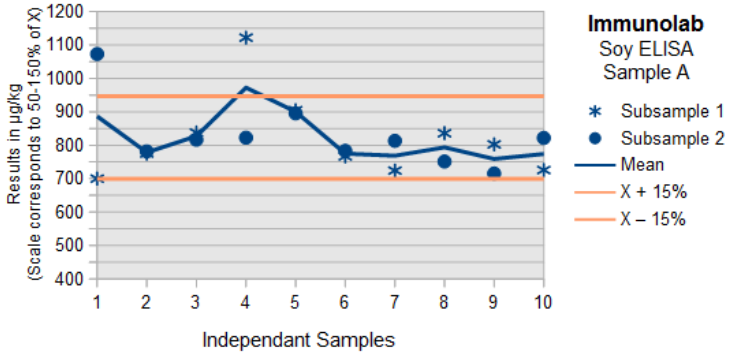
ELISA-Tests: Homogenität Soja / Homogeneity Soya

Immunolab Soy ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soy trypsin inhibitor 823 ± 44 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	700	1073	886
2	775	782	778
3	838	816	827
4	1122	823	972
5	905	895	900
6	767	783	775
7	724	813	769
8	836	751	794
9	803	716	759
10	726	822	774

General average X	823	
SD of sample means Sx	72,1	8,8%
SD within-samples Sw	80,7	9,8%
SD between-samples Ss	44,1	5,3%

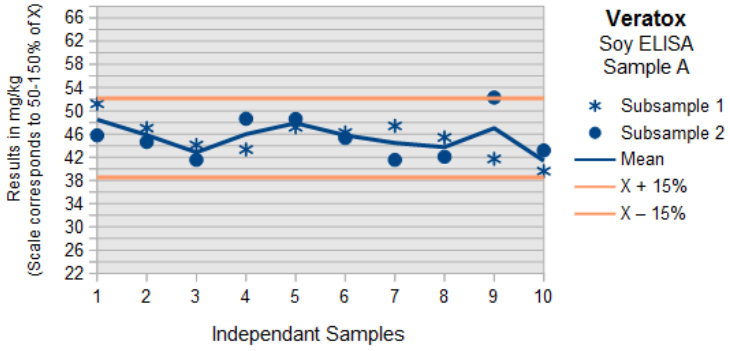


Neogen Veratox ELISA Soy

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Soyflour 45,3 ± 1,4 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	51,2	45,8	48,5
2	47,0	44,6	45,8
3	44,1	41,6	42,8
4	43,3	48,6	46,0
5	47,2	48,6	47,9
6	46,3	45,3	45,8
7	47,4	41,5	44,5
8	45,4	42,1	43,8
9	41,7	52,3	47,0
10	39,6	43,2	41,4

General average X	45,3	
SD of sample means Sx	2,24	4,9%
SD within-samples Sw	2,46	5,4%
SD between-samples Ss	1,41	3,1%

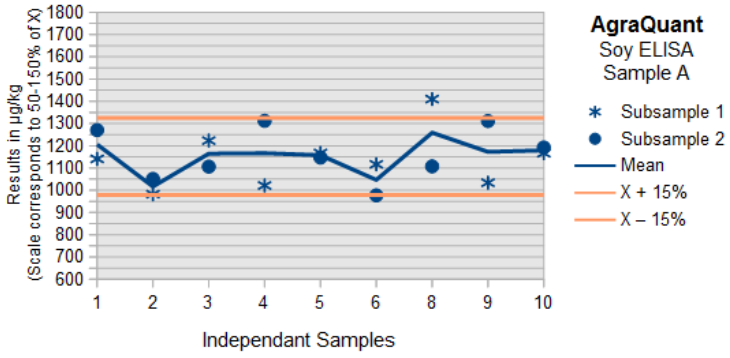


Romerlabs AgraQuant Soy

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soy trypsin inhibitor 1150 ± 37 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	1141	1270	1206
2	982	1051	1017
3	1223	1106	1165
4	1022	1312	1167
5	1168	1147	1157
6	1117	977	1047
8	1410	1108	1259
9	1034	1311	1173
10	1166	1192	1179

General average X	1152	
SD of sample means Sx	75,1	6,5%
SD within-samples Sw	92,5	8,0%
SD between-samples Ss	36,9	3,2%



2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,14-0,18 (22-24°C) The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A, B and the spiking level sample were sent to every participating laboratory in the 4th week of 2019. The testing method was optional. The tests should be finished at 8th March 2019 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 Soya in the range of mg/kg in the matrix of Sauce Powder. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing. Note: The matrix sample provided as a blank sample (A or B) can not be guaranteed to be "allergen-free".

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 24 participants submitted their results in time.

3. Evaluation

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

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

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

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

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

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^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, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 15 – 43% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

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

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

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-35]

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 saus-age (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 saus-age (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
Wheat + Rye	Boiled saus-age (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.

An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) 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 can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.8 Standard uncertainty and traceability

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

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

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures

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

3.10 Recovery rates: Spiking

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

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.

Casein-specific ELISA results reported as **skimmed milk powder** have been converted to **casein**. For this purpose, the specifications of the respective test kit manufacturer for the casein content in skimmed milk powder were taken into account (AgraQuant Casein ELISA Test Kit Manual: conversion factor 3.6 (27.8%)).

Casein-specific ELISA results, given as **milk protein (total)**, have also been converted into **casein** using a content of 80% casein in total milk protein from the literature [36].

Milk protein-specific ELISA results reported as **skimmed milk powder** have been converted to **total milk protein**.
As far as available, the specifications of the respective test kit manufacturer for the content of total milk protein in skimmed milk powder were taken into account (Neogen Allergen Handbook: 35.1%). The results of the other methods were converted into the total milk protein using the experimentally determined protein content of the raw material (see p.5).

Milk protein-specific ELISA results reported as the sum of **casein and β-lactoglobulin** (SensiSpec ELISA) have not been converted, but have been equated with total milk protein. (Note: the conversion factor of 2.7 for skimmed milk powder from the kit manual and the total experimental milk protein content of the raw material would otherwise result in a lower content than originally stated).

The ELISA results reported as **soyflour / soybean** have been converted to **soy protein**. As far as available, the specifications of the respective test kit manufacturer for the content of total protein in soyflour were taken into account (Neogen Allergen Handbook: 47.01%). The results of the other methods were converted into soy protein using the experimentally determined protein content of the raw material (see p.5). (see p. 5).

The ELISA results reported as soybean trypsin inhibitor (STI) were evalu-

ated separately as such.

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		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* σ_{pt}'		
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}'$)°		
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}'$)°		
Quotient S*/σ_{pt} *or* S*/σ_{pt}'		
Standard uncertainty $U_{(X_{pt})}$		
Number of results in target range		
Percent in target range		

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

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

4.1 Proficiency Test Milk

4.1.1 ELISA Results: Milk (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		
1	positive	13,3	positive	8,30	2/2 (100%)	AQ	
4	positive	21,7	positive	14,4	2/2 (100%)	AQ	Result converted °
6	positive	12,3	positive	8,71	2/2 (100%)	AQ	
15	positive	10,2	positive	6,60	2/2 (100%)	AQ	
19	positive	18,8	positive	14,6	2/2 (100%)	AQ	
7	positive	16,5	positive	13,8	2/2 (100%)	BF	Result converted °
16	positive	18,5	positive	14,0	2/2 (100%)	EF	
5	positive	14,9	positive	13,0	2/2 (100%)	IL	
8	positive	47,5	positive	29,8	2/2 (100%)	MI	Result converted °
18	positive	34,0	positive	28,0	2/2 (100%)	MI-II	
12	positive	19,0	positive	13,0	2/2 (100%)	NL	
9	positive	43,9	positive	32,5	2/2 (100%)	RS-F	
11	positive	14,0	positive	7,30	2/2 (100%)	RS-F	
24	positive	35,0	positive	25,0	2/2 (100%)	RS-F	

° calculation see p. 19

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

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI = Morinaga Institute ELISA
MI-II = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
The consensus value of sample A is in qualitative agreement with the spiking of sample A.
Sample B without spiked allergens contains also milk from the matrix instant sauce powder. Consistently, the participants obtained a consensus value of 100% positive results.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Casein	z'-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
1	13,3	-1,3	-0,5	AQ	
4	21,7	0,0	1,7	AQ	Result converted °
6	12,3	-1,4	-0,8	AQ	
15	10,2	-1,7	-1,3	AQ	
19	18,8	-0,5	0,9	AQ	
7	16,5	-0,8		BF	Result converted °
16	18,5	-0,5		EF	
5	14,9	-1,1		IL	
8	47,5	3,8		MI	Result converted °
18	34,0	1,7		MI-II	
12	19,0	-0,4		NL	
9	43,9	3,2		RS-F	
11	14,0	-1,2		RS-F	
24	35,0	1,9		RS-F	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

MI = Morinaga Institute ELISA

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

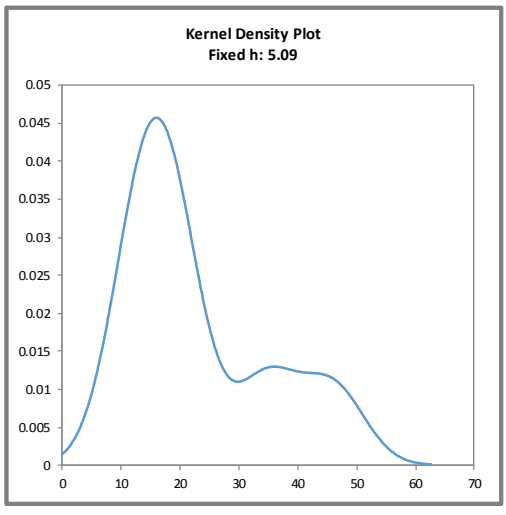


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with an additional broad peak with two maxima between 30-60 mg/kg due to some results of the methods MI, MI-II and RS-F.

Characteristics: Quantitative evaluation ELISA Casein

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	14	5
Number of outliers	0	0
Mean	22,8	15,3
Median	18,7	13,3
Robust Mean (X_{pt})	**22,0**	**15,3**
Robust standard deviation (S*)	**11,9**	**5,45**
Target range:		
Target standard deviation $\sigma_{pt'}$ and σ_{pt}	**6,79**	**3,82**
lower limit of target range	**8,41**	**7,63**
upper limit of target range	**35,6**	**22,9**
Quotient S/$\sigma_{pt'}$ and S*/σ_{pt}*	*1,8*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*3,99*	*3,05*
Results in the target range	*12*	*5*
Percent in the target range	*86*	*100*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (higher values are based on individual results).

The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} of > 2,0. Therefore the evaluation of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then below 2,0.*
The evaluation of the method AQ showed a normal variability of results. The quotient S/σ_{pt} was below 2,0.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluation of all results and method AQ were 64% and 44% of the spiking level of casein to sample A and within or just below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.35).

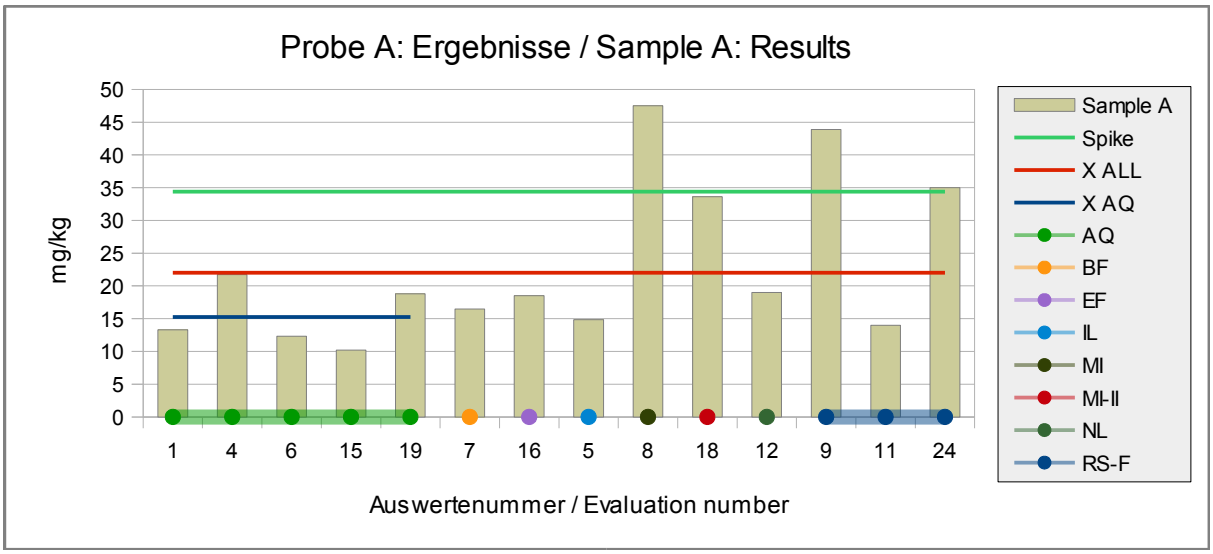


Abb./Fig. 2: ELISA Results Milk (casein)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

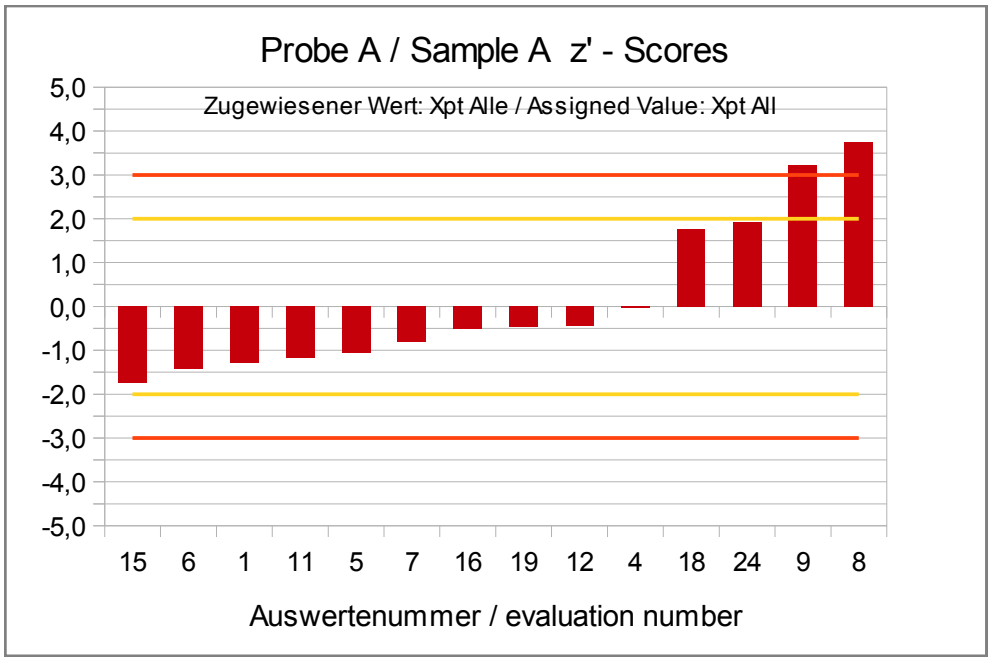


Abb./Fig. 3:
z'-Scores (ELISA Results casein)
Assigned value robust mean of all results

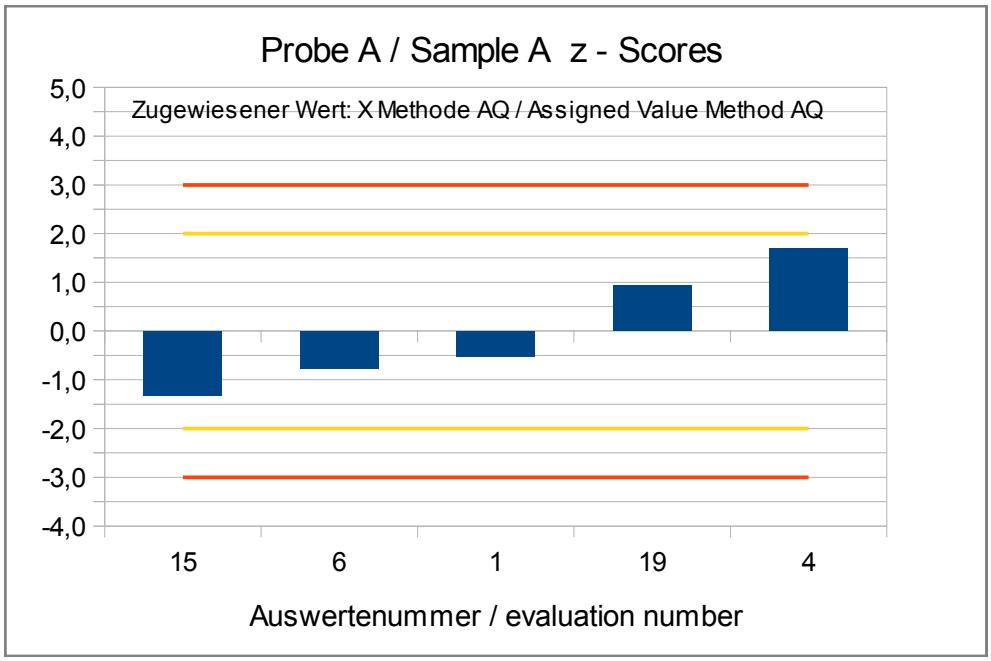


Abb./Fig. 4:
z-Scores (ELISA Results casein)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Casein	z'-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
1	8,30	-1,5	-0,8	AQ	
4	14,4	-0,4	1,5	AQ	Result converted °
6	8,71	-1,5	-0,7	AQ	
15	6,60	-1,9	-1,5	AQ	
19	14,6	-0,3	1,6	AQ	
7	13,8	-0,5		BF	Result converted °
16	14,0	-0,4		EF	
5	13,0	-0,6		IL	
8	29,8	2,6		MI	Result converted °
18	28,0	2,3		MI-II	
12	13,0	-0,6		NL	
9	32,5	3,2		RS-F	
11	7,30	-1,7		RS-F	
24	25,0	1,7		RS-F	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

MI = Morinaga Institute ELISA

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

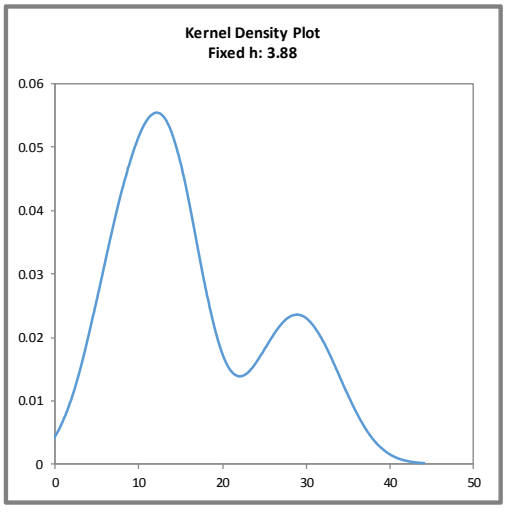


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with an additional peak at approx. 30 mg/kg due to some results of the methods MI, MI-II and RS-F.

Characteristics: Quantitative evaluation ELISA Casein

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	14	5
Number of outliers	0	0
Mean	16,4	10,5
Median	13,9	8,71
Robust Mean (X_{pt})	**16,2**	**10,5**
Robust standard deviation (S*)	**9,63**	**4,22**
Target range:		
Target standard deviation $\sigma_{pt'}$ and σ_{pt}	**5,17**	**2,63**
lower limit of target range	**5,87**	**5,26**
upper limit of target range	**26,6**	**15,8**
Quotient S/$\sigma_{pt'}$ and S*/σ_{pt}*	*1,9*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*3,22*	*2,36*
Results in the target range	*11*	*5*
Percent in the target range	*79*	*100*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (higher values are based on individual results).

The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} above 2,0. Therefore the evaluation of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then below 2,0.*
The evaluation of the method AQ showed a normal variability of results. The quotient S/σ_{pt} was below 2,0.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

Sample B was not spiked with allergens. Therefore no recovery rates for sample B could be calculated. The contents come from the matrix instant sauce powder.

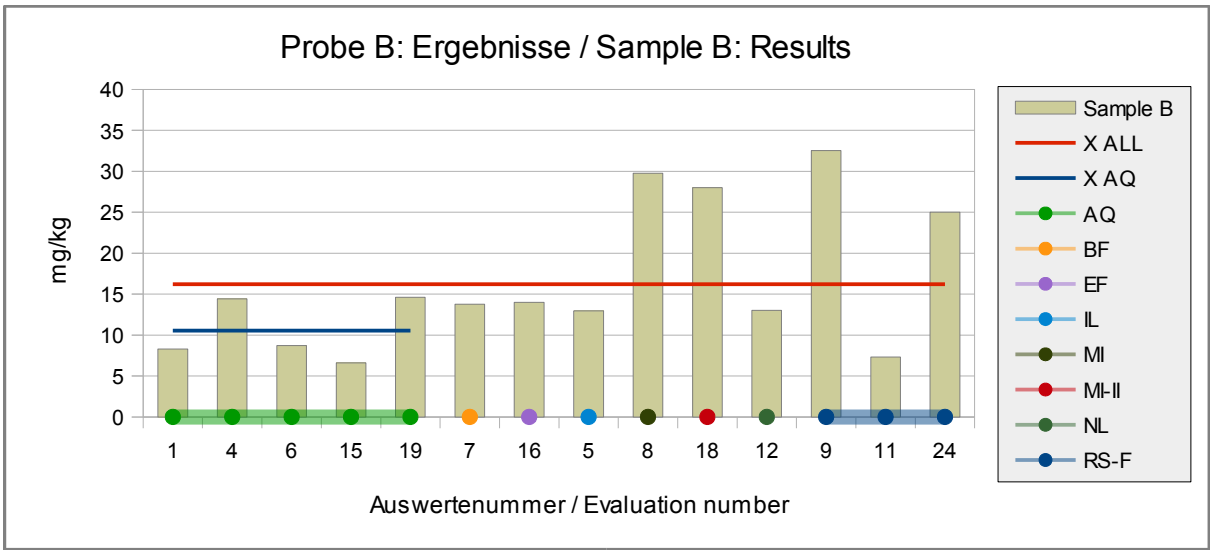


Abb./Fig. 6: ELISA Results Milk (casein)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

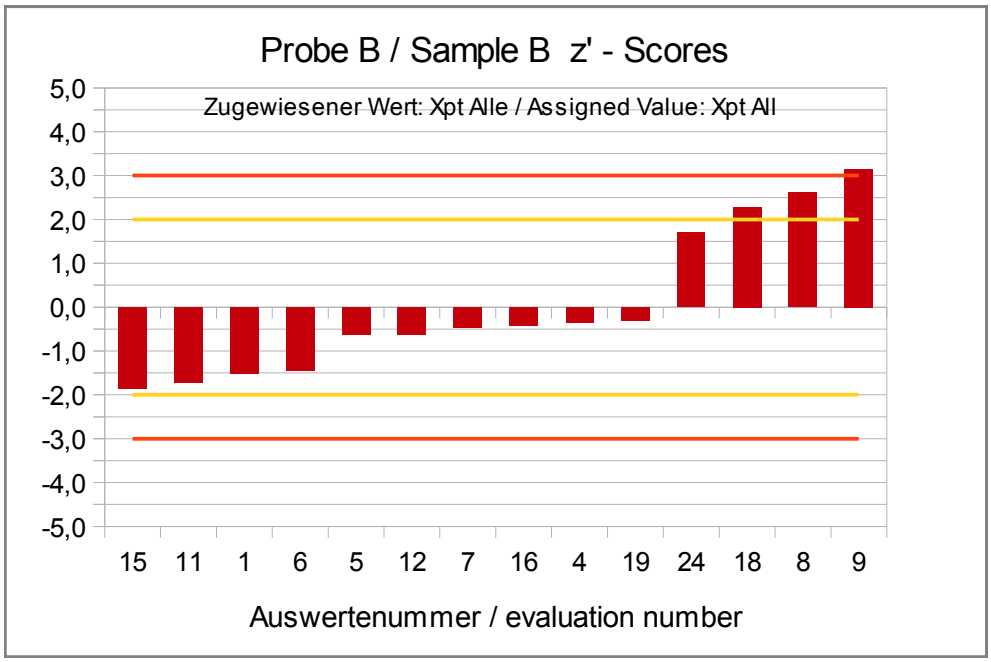


Abb./Fig. 7:
z'-Scores (ELISA Results casein)
Assigned value robust mean of all results

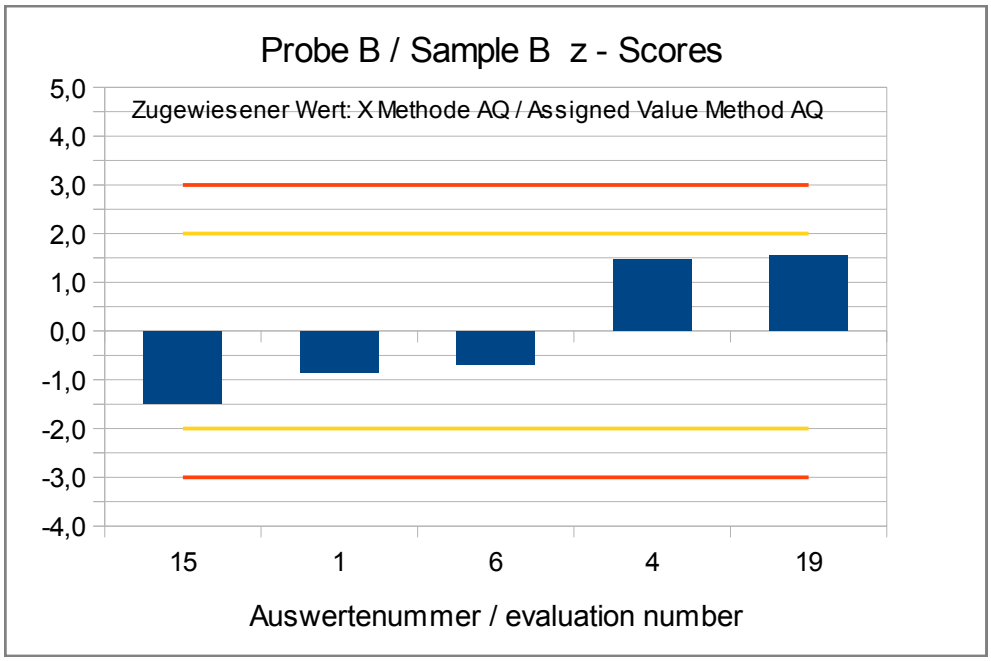


Abb./Fig. 8:
z-Scores (ELISA Results casein)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Quantitative valuation of results: Spiking level sample

	Casein	z-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
1	22,5	0,8	0,7	AQ	
4	22,5	0,8	0,7	AQ	Result converted °
6	16,0	-0,6	-0,7	AQ	
15	11,6	-1,5	-1,6	AQ	
19	23,4	1,0	0,9	AQ	
7	18,2	-0,1		BF	Result converted °
16	17,6	-0,3		EF	
5	26,4	1,6		IL	
8	18,8	0,0		MI	Result converted °
18	14,0	-1,0		MI-II	
12	21,0	0,5		NL	
9	22,5	0,8		RS-F	
11	4,40	-3,1		RS-F	
24	18,0	-0,2		RS-F	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI = Morinaga Institute ELISA
MI-II = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm

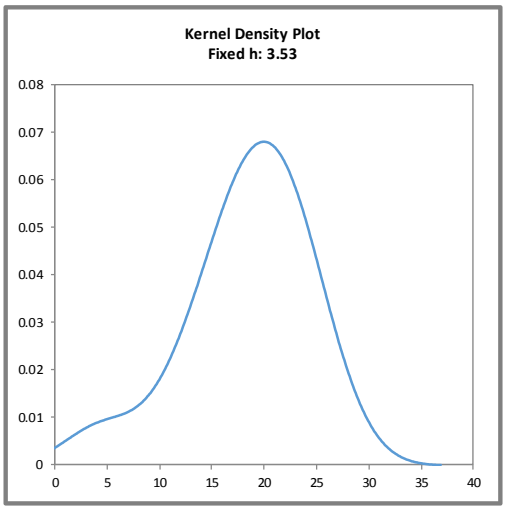


Abb. / Fig. 9:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 symmetrical distribution of results with a smaller shoulder at approx. 5 mg/kg due to a single result (method RS-F).

Characteristics: Quantitative evaluation ELISA Casein

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	14	5
Number of outliers	0	0
Mean	18,3	19,2
Median	18,5	22,5
Robust Mean (X)	**18,8**	**19,2**
Robust standard deviation (S*)	**5,15**	**5,88**
Target range:		
Target standard deviation σ_{pt}	**4,71**	**4,80**
lower limit of target range	**9,41**	**9,60**
upper limit of target range	**28,2**	**28,8**
Quotient S/σ_{pt}*	*1,1*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*1,72*	*3,29*
Results in the target range	*13*	*5*
Percent in the target range	*93*	*100*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (a high single value).

The evaluation of all methods and the evaluation of results from method AQ showed a low to normal 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 only a few results for some methods.*

The robust means of the evaluation of all results and method AQ were 134% and 136% of the spiking level of casein to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.35).

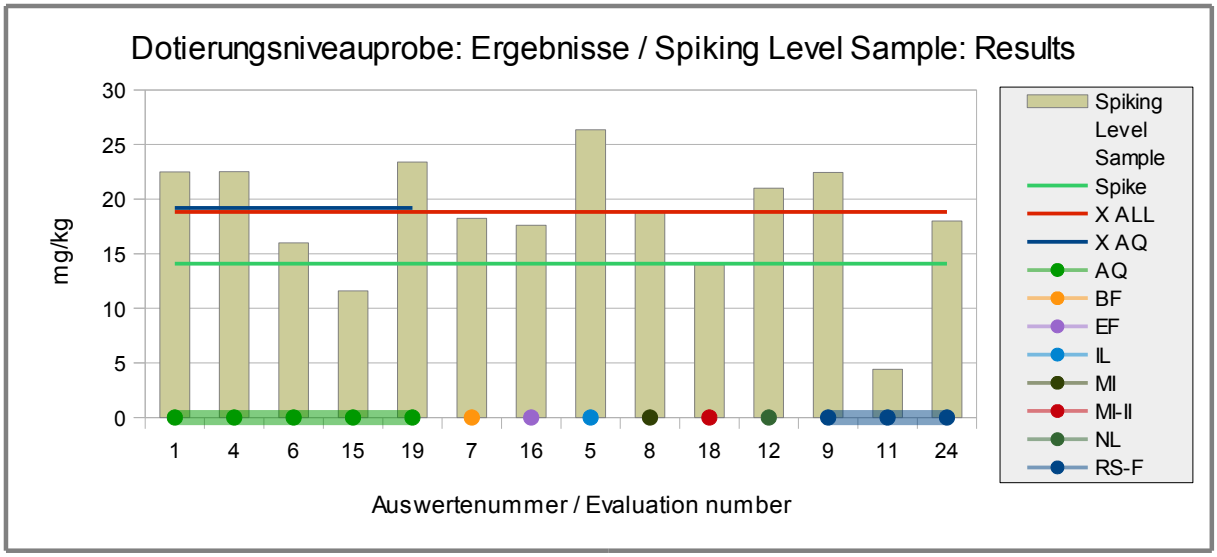


Abb./Fig. 10: ELISA Results casein
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

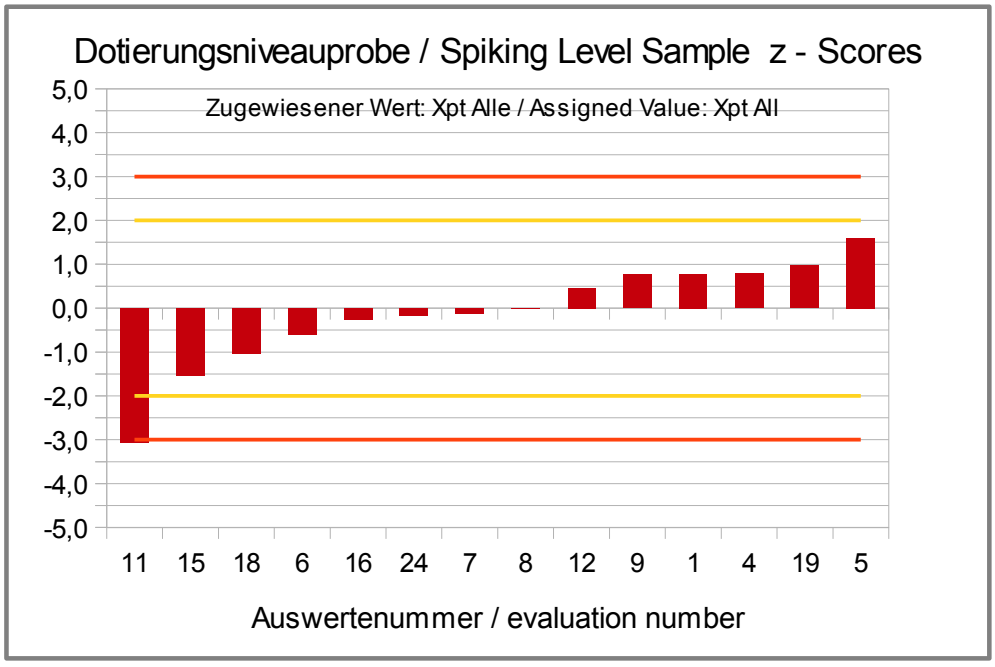


Abb./Fig. 11:
z-Scores (ELISA results casein)
Assigned value robust mean of all results

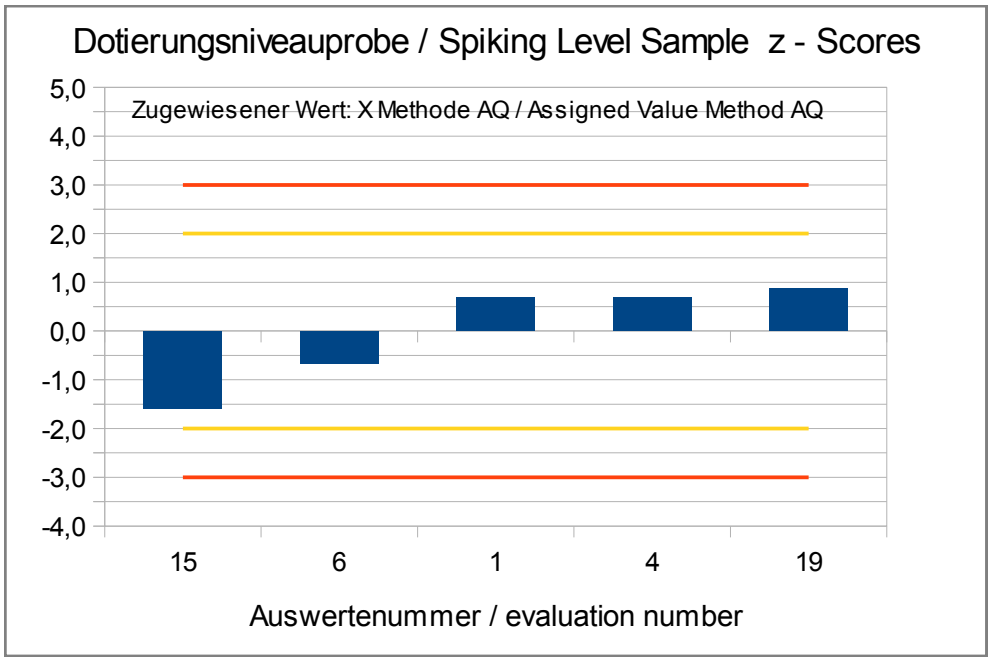


Abb./Fig. 12:
z-Scores (ELISA Results casein)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Recovery Rates ELISA for Casein: Spiking level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	22,5	160	13,3	39	AQ	
4	22,5	160	21,7	**63**	AQ	Result converted °
6	16,0	**113**	12,3	36	AQ	
15	11,6	**82**	10,2	30	AQ	
19	23,4	166	18,8	**55**	AQ	
7	18,2	**129**	16,5	48	BF	Result converted °
16	17,6	**125**	18,5	**54**	EF	
5	26,4	187	14,9	43	IL	
8	18,8	**133**	47,5	**138**	MI	Result converted °
18	14,0	**99**	34,0	**99**	MI-II	
12	21,0	**149**	19,0	**55**	NL	
9	22,5	159	43,9	**128**	RS-F	
11	4,40	31	14,0	41	RS-F	
24	18,0	**128**	35,0	**102**	RS-F	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	8	Number in RA	8
Percent in RA	57	Percent in RA	57

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

MI = Morinaga Institute ELISA

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

In each case, 57% (8) of the participants obtained for the spiking level sample and for the spiked processed food matrix sample A with ELISA recovery rates within the range of the AOAC-recommendation of 50-150%.

To calculate the nominal values for sample A:

Besides the addition of skimmed milk powder (see page 5), sample A contains total milk protein or casein from the matrix instant sauce powder. For the calculation of the recovery rates of the participants' results, therefore, the average contents of total milk protein and casein from sample B were taken into account. The results for sample B were set as 100% content in the matrix, the added content to sample A then corresponds to about another 41%. This results in the sum of the following nominal values which were used for the calculation of the recovery rate for sample A: total milk protein 44.0 mg/kg and casein 34.4 mg/kg.

4.1.2 ELISA Results: Milk Protein, total

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		
4	positive	19,2	positive	11,1	2/2 (100%)	AQ	Result converted °
12a	positive	27,0	positive	16,0	2/2 (100%)	AQ	
19	positive	16,9	positive	12,9	2/2 (100%)	AQ	
20	positive	20,0	positive	15,1	2/2 (100%)	AQ	
16	positive	21,7	positive	10,8	2/2 (100%)	EF	sum of β-Lactoglobulin + Casein
23	positive	25,0	positive	15,0	2/2 (100%)	EF	sum of β-Lactoglobulin + Casein
8	positive	54,5	positive	26,6	2/2 (100%)	MI-II	
12b	positive	26,0	positive	16,0	2/2 (100%)	NL	
5	positive	55,7	positive	37,9	2/2 (100%)	RS-F	
9	positive	67,9	positive	42,3	2/2 (100%)	RS-F	
11	positive	109	positive	60,0	2/2 (100%)	RS-F	
21	positive	47,9	positive	32,5	2/2 (100%)	RS-F	
3	positive	>25.0	positive	>25.0	2/2 (100%)	VT	
6	positive	33,3	positive	19,1	2/2 (100%)	VT	Result converted °
17	positive	38,6	positive	32,3	2/2 (100%)	VT	Result converted °

° calculation see p. 19

	Sample A		Sample B	
Anzahl positiv	15		15	
Anzahl negativ	0		0	
Prozent positiv	100		100	
Prozent negativ	0		0	
Konsenswert	positive		positive	

Methods:

AQ = AgraQuant, RomerLabs
EF = SensiSpec ELISA Kit, Eurofins
MI-II = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS-F = Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus value of sample A is in qualitative agreement with the spiking of sample A.
Sample B without spiked allergens contains also milk from the matrix instant sauce powder. Consistently, the participants obtained a consensus value of 100% positive results.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Milk protein	z-Score Xpt_{25}	z-Score Xpt56	Method	Remarks
	[mg/kg]				
4	19,2	-0,9		AQ	Result converted °
12a	27,0	0,3		AQ	
19	16,9	-1,3		AQ	
20	20,0	-0,8		AQ	
16	21,7	-0,5		EF	sum of β-Lactoglobulin + Casein
23	25,0	0,0		EF	sum of β-Lactoglobulin + Casein
8	54,5		-0,1	MI-II	
12b	26,0	0,2		NL	
5	55,7		0,0	RS-F	
9	67,9		0,9	RS-F	
11	109		3,8	RS-F	
21	47,9		-0,6	RS-F	
3	>25.0			VT	
6	33,3	1,3		VT	Result converted °
17	38,6	2,2		VT	Result converted °

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs

EF = SensiSpec ELISA Kit, Eurofins

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F = Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

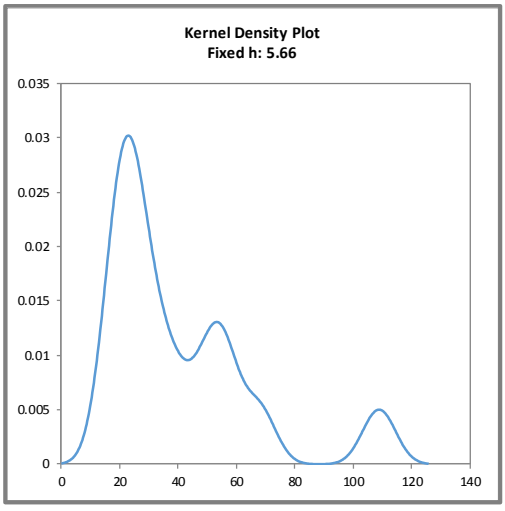


Abb. / Fig. 13:

Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:

The kernel density estimation shows a major peak at approx. 25 mg/kg with a nearly symmetric distribution of results. Furthermore, a secondary peak occurs at about 55 mg/kg with a shoulder and a smaller peak at about 110 mg/kg, which is due to a single value. The higher values are based on the results of the methods RS-F and MI-II and were therefore evaluated separately.

Characteristics: Quantitative evaluation ELISA Milk (as Milk Protein)

Sample A

Statistic Data	**Meth. Peak 25** *[mg/kg]*	**Meth. Peak 56** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{25}}}$	$\boldsymbol{X_{pt_{56}}}$
Number of results	9	5
Number of outliers	0	0
Mean	25,3	67,0
Median (X_{pt})⁺⁺	25,0	**55,7**
Robust Mean (X_{pt})⁺	**25,0**	65,8
Robust standard deviation (S*)	**7,26**	**20,0**
Target range:		
Target standard deviation σ_{pt}	**6,25**	**13,9**
lower limit of target range	**12,5**	**27,9**
upper limit of target range	**37,5**	**83,6**
Quotient S/σ_{pt}*	*1,2*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*3,02*	*11,2*
Results in the target range	*8*	*4*
Percent in the target range	*89*	*80*

⁺ Assigned value (*Xpt*) for peak 25: rob. Mean

⁺⁺ Assigned value (Xpt) for peak 56: Median

Method:
Peak 25 = AgraQuant, SensiSpec , nutriLinia®, Veratox
Peak 56 = Morinaga, Ridascreen Fast®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution of results with a main peak and a minor peak (as well as a further minor peak of a single result). Therefore, no common evaluation of all methods was done, but two evaluations separated according to methods that can be assigned to the first peak (peak 25) and the second peak (peak 56) (assignment see above under the table).

The evaluation of the results of peak 25 and the evaluation of results of peak 56 showed a normal 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 only a few results for some methods.*

The robust mean of the evaluation of the results of peak 25 was with 57% of the spiking level of milk protein to sample A and within the range of the recommendations for the applied methods. The median of the evalu-

ation of the results of peak 56 was with 127% also within the range of the recommendations for the applied methods(s. 3.4.3 and "Recovery rates of milk protein" p.49).

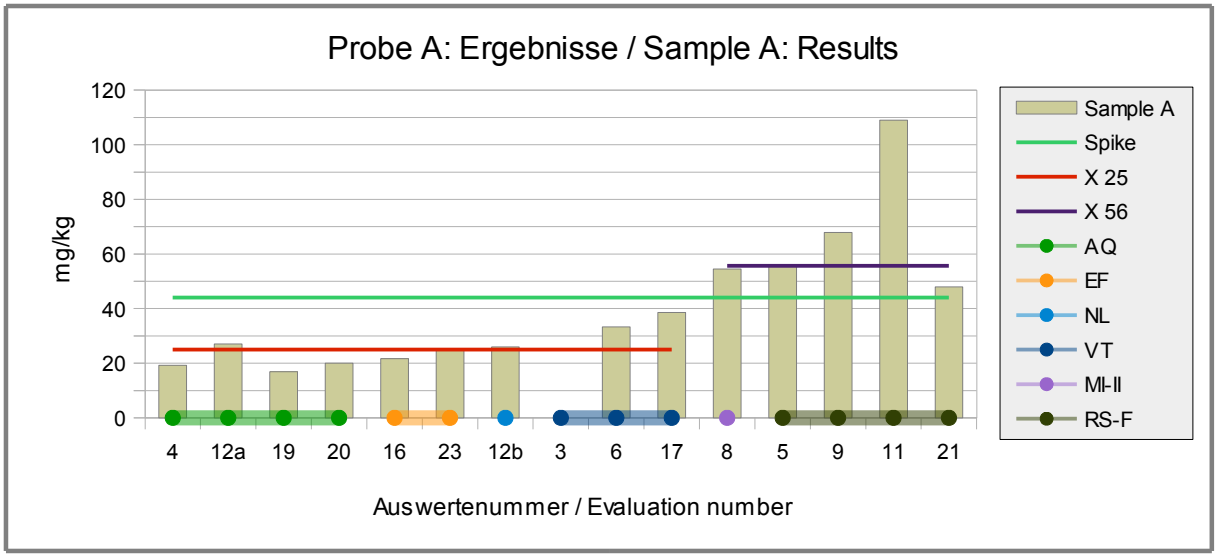


Abb./Fig. 14: ELISA Results Milk (milk protein)
green line = Spiking level
red line = Assigned value robust mean results peak 25
blue line = Assigned value median results peak 56
round symbols = Applied methods (see legend)

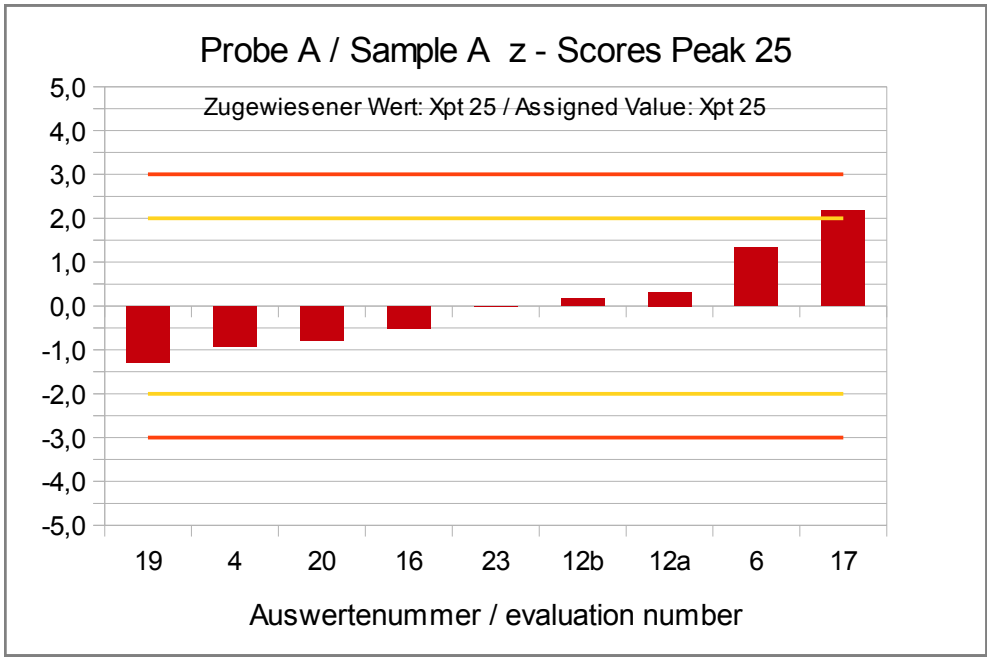


Abb./Fig. 15:
z-Scores (ELISA Results milk as milk protein)
Assigned value robust mean of all results of peak 25

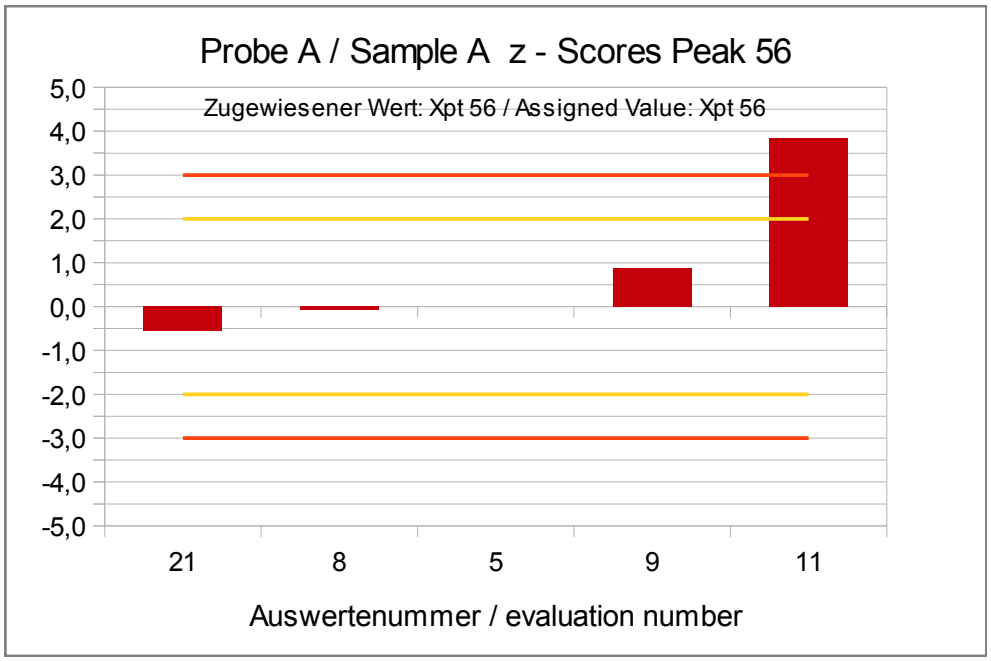


Abb./Fig. 16:
z-Scores (ELISA Results milk as milk protein)
Assigned value median of all results of peak 56

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Milk protein	z-Score Xpt_{15}	z-Score Xpt_{40}	Method	Remarks
	[mg/kg]				
4	11,1	-1,1		AQ	Result converted °
12a	16,0	0,2		AQ	
19	12,9	-0,6		AQ	
20	15,1	0,0		AQ	
16	10,8	-1,2		EF	sum of β-Lactoglobulin + Casein
23	15,0	-0,1		EF	sum of β-Lactoglobulin + Casein
8	26,6		-1,3	MI-II	
12b	16,0	0,2		NL	
5	37,9		-0,2	RS-F	
9	42,3		0,2	RS-F	
11	60,0		2,0	RS-F	
21	32,5		-0,7	RS-F	
3	>25,0			VT	
6	19,1	1,0		VT	Result converted °
17	32,3	4,5		VT	Result converted °

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
EF = SensiSpec ELISA Kit, Eurofins
MI-II = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS-F = Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

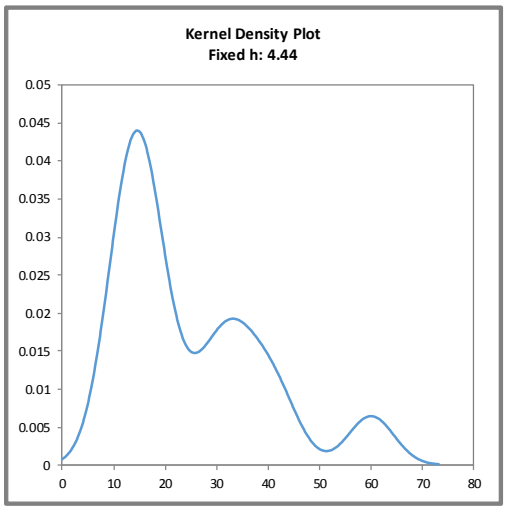


Abb. / Fig. 17:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a major peak at approx. 15 mg/kg with a nearly symmetric distribution of results. Furthermore, a secondary peak occurs at about 30-40 mg/kg and a smaller peak at about 60 mg/kg, which is due to a single result. The higher values are based on the results of the methods RS-F and MI-II and were therefore evaluated separately.

Characteristics: Quantitative evaluation ELISA Milk (as Milk Protein)

Sample B

Statistic Data	**Meth. Peak 15** *[mg/kg]*	**Meth. Peak 40** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{15}}}$	$\boldsymbol{X_{pt_{40}}}$
Number of results	9	5
Number of outliers	-	0
Mean	16,5	39,9
Median	15,1	37,9
Robust Mean (X_{pt})	**15,2**	**39,9**
Robust standard deviation (S*)	**3,85**	**14,4**
Target range:		
Target standard deviation σ_{pt}	**3,81**	**9,96**
lower limit of target range	**7,61**	**19,9**
upper limit of target range	**22,8**	**59,8**
Quotient S/σ_{pt}*	*1,0*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*1,61*	*8,05*
Results in the target range	*8*	*4*
Percent in the target range	*89*	*80*

Method:
Peak 15 = AgraQuant, SensiSpec , nutriLinia®, Veratox
Peak 40 = Morinaga, Ridascreen Fast®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution of results with a main peak and a minor peak (as well as a further minor peak of a single result). Therefore, no common evaluation of all methods was done, but two evaluations separated according to the methods that can be assigned to the first peak (peak 15) and the second peak (peak 40) (assignment see above under the table).

The evaluation of the results of peak 15 and the evaluation of results of peak 35 showed a normal 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 only a few results for some methods.*

Sample B was not spiked with allergens. Therefore no recovery rates for sample B could be calculated. The contents come from the matrix instant sauce powder.

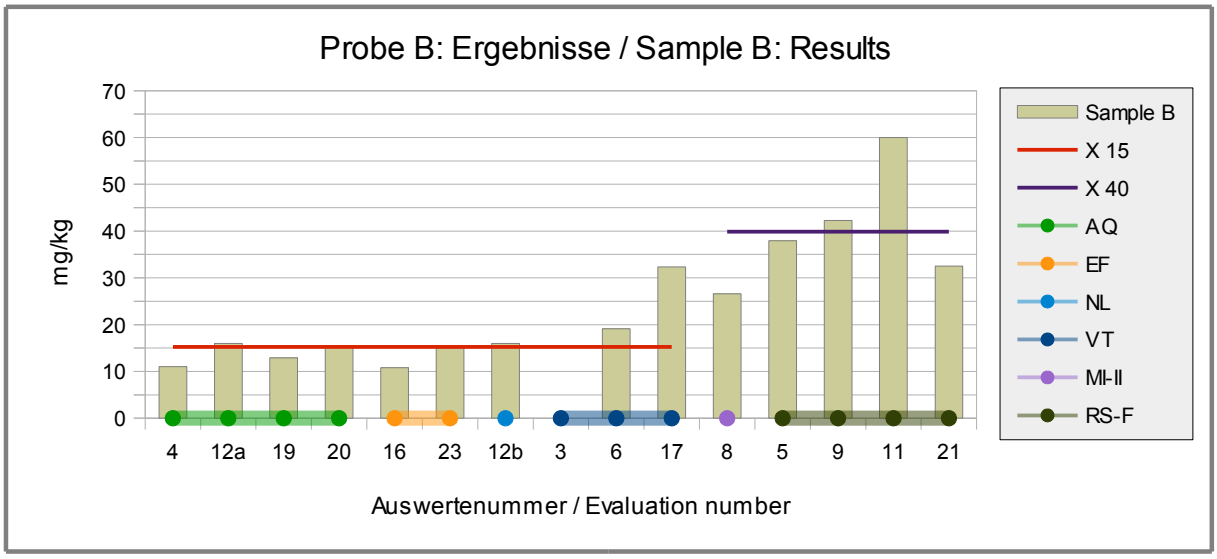


Abb./Fig. 18: ELISA Results Milk (as milk protein)
red line = robust mean peak 15
blue line = robust mean peak 40
round symbols = Applied methods (see legend)

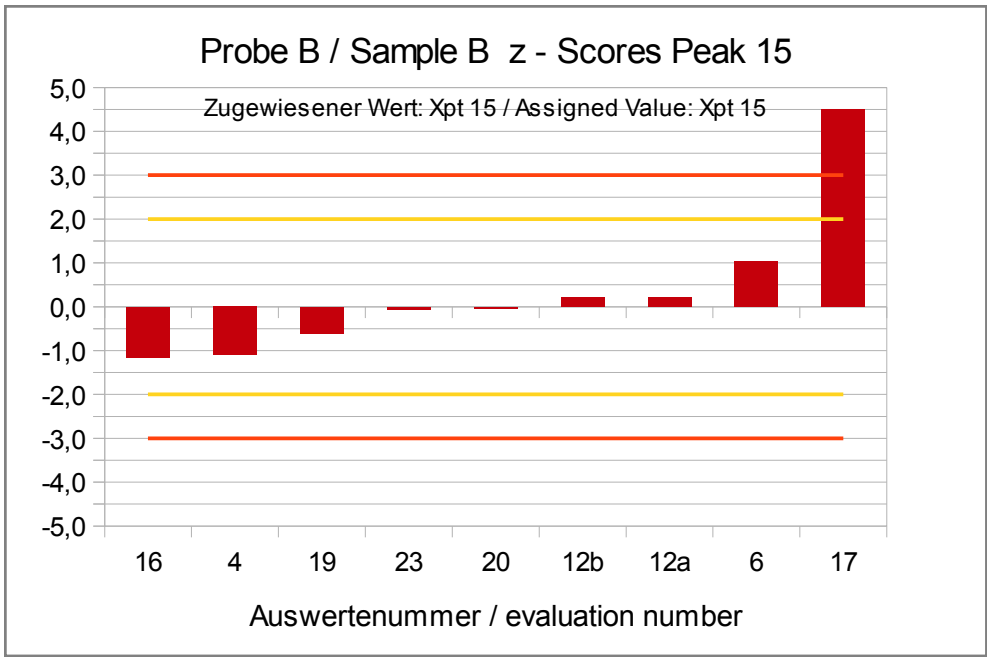


Abb./Fig. 19:
z-Scores (ELISA Results milk as milk protein)
Assigned value robust mean of all results of peak 15

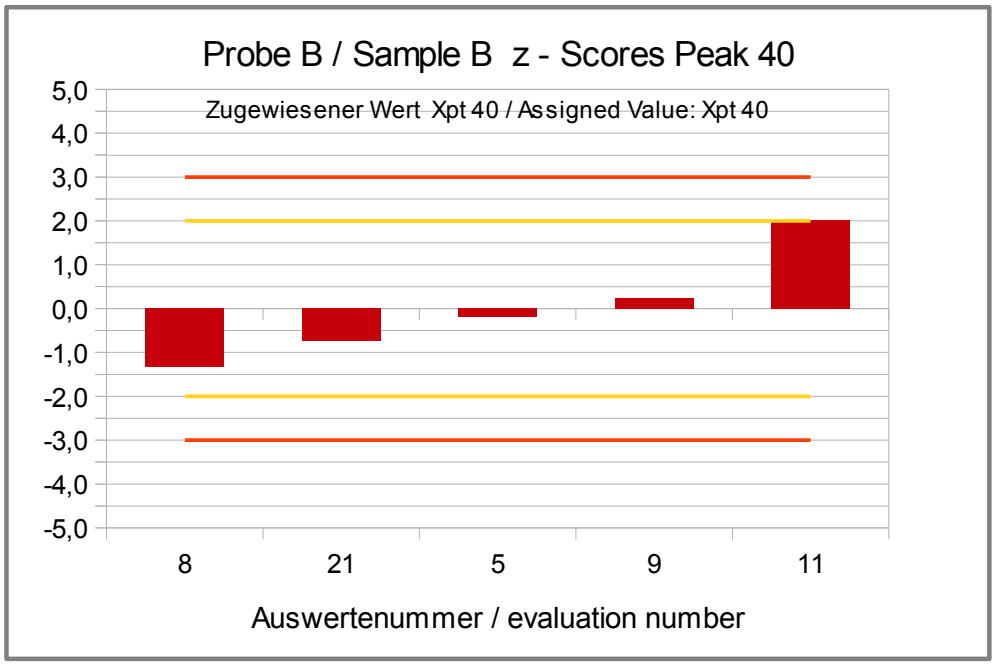


Abb./Fig. 20:
z-Scores (ELISA Results milk as milk protein)
Assigned value robust mean of all results of peak 40

Quantitative valuation of results: Spiking level sample

Evaluation number	Milk Protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4	20,1	-0,1	AQ	Result converted °
12a	27,0	1,2	AQ	
19	22,0	0,3	AQ	
20	17,8	-0,6	AQ	
16	35,9	2,9	EF	sum of β-Lactoglobulin + Casein
23	25,0	0,8	EF	sum of β-Lactoglobulin + Casein
8	16,5	-0,8	MI-II	
12b	36,0	3,0	NL	
5	16,8	-0,8	RS-F	
9	20,7	0,0	RS-F	
11	57,0		RS-F	outlier excluded
21	11,9	-1,7	RS-F	
3			VT	
6	7,13	-2,6	VT	Result converted °
17	15,8	-0,9	VT	Result converted °

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs

EF = SensiSpec ELISA Kit, Eurofins

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F = Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

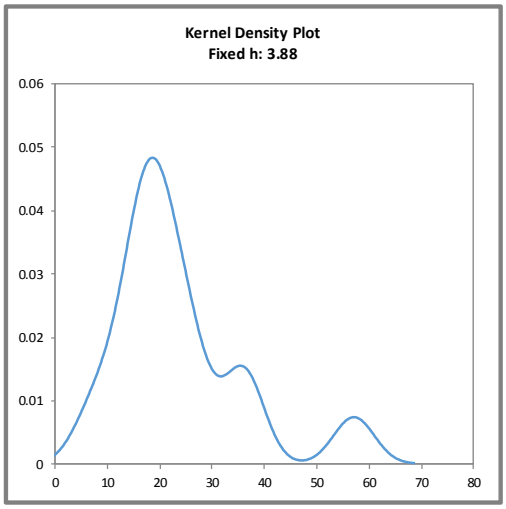


Abb. / Fig. 21:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a shoulder at approx. 35 mg/kg and a smaller peak at approx. 57 mg/kg due to an outlier (method RS-F) .

Characteristics: Quantitative evaluation ELISA Milk (as Milk Protein)

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	13
Number of outliers	1
Mean	21,0
Median	20,1
Robust Mean (X)	**20,7**
Robust standard deviation (S*)	**8,85**
Target range:	
Target standard deviation σ_{pt}	**5,17**
lower limit of target range	**10,3**
upper limit of target range	**31,0**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*3,07*
Results in the target range	*10*
Percent in the target range	*77*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences.

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 given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust mean of the evaluation of all results was 118% of the spiking level of milk protein to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of milk protein" p.49).

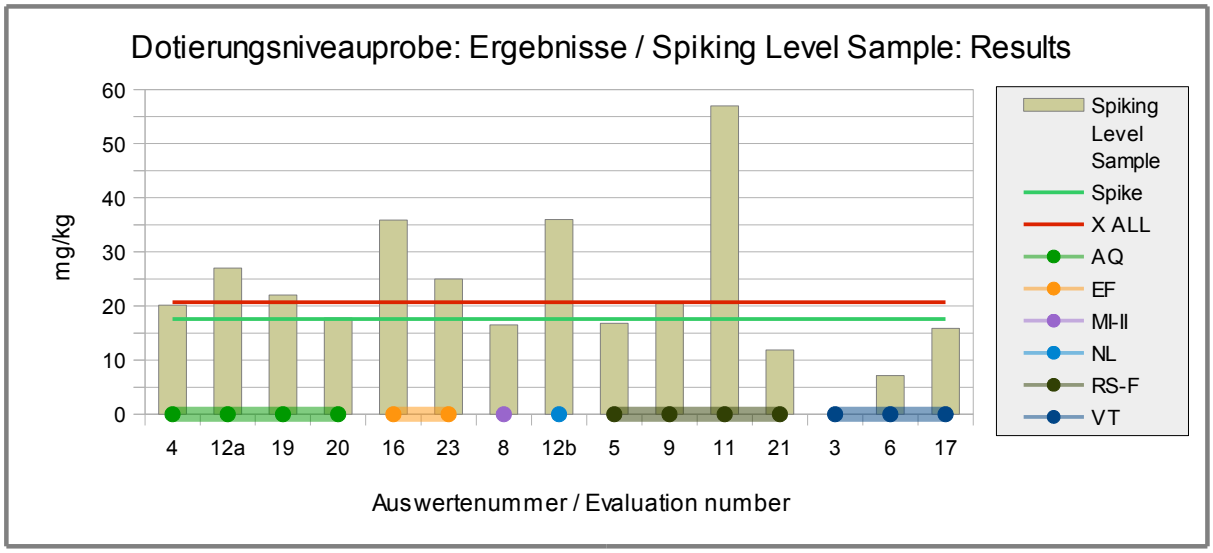


Abb./Fig. 22: ELISA Results milk (as milk protein)
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

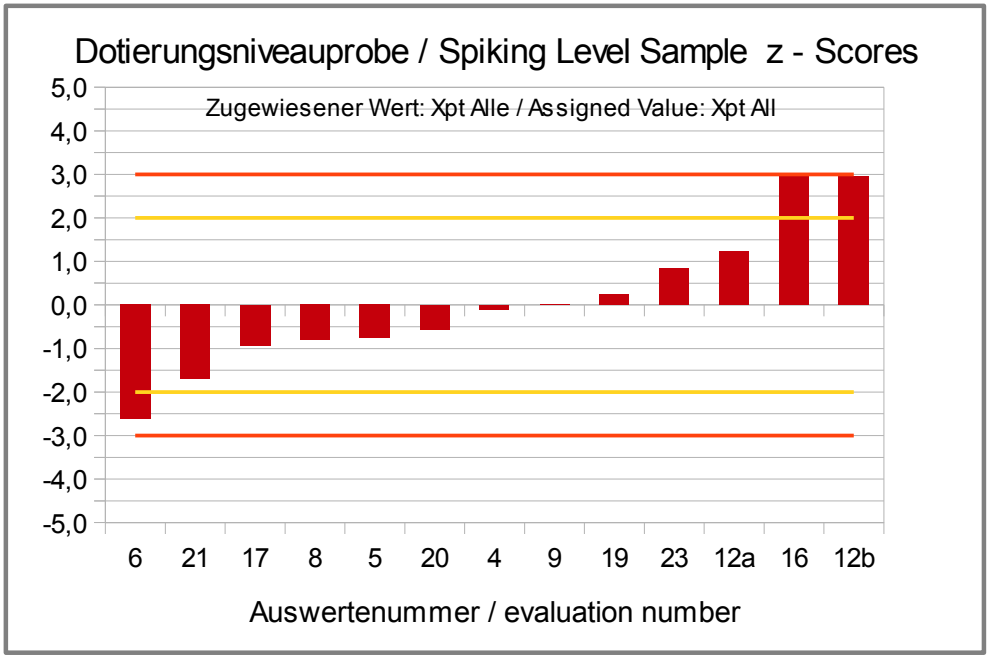


Abb./Fig. 23:
z-Scores (ELISA results milk as milk protein)
Assigned value robust mean of all results

Recovery Rates ELISA for Milk Protein: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4	20,1	**114**	19,2	44	AQ	Result converted °
12a	27,0	153	27,0	**61**	AQ	
19	22,0	**125**	16,9	38	AQ	
20	17,8	**101**	20,0	45	AQ	
16	35,9	204	21,7	49	EF	sum of β-Lactoglobulin + Casein
23	25,0	**142**	25,0	**57**	EF	sum of β-Lactoglobulin + Casein
8	16,5	**94**	54,5	**124**	MI-II	
12b	36,0	205	26,0	**59**	NL	
5	16,8	**95**	55,7	**127**	RS-F	
9	20,7	**117**	67,9	154	RS-F	
11	57,0	324	109	248	RS-F	
21	11,9	**68**	47,9	**109**	RS-F	
3			>25.0		VT	
6	7,13	41	33,3	**76**	VT	Result converted °
17	15,8	**90**	38,6	**88**	VT	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	9	Number in RA	8
Percent in RA	64	Percent in RA	57

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

EF = SensiSpec ELISA Kit, Eurofins

MI-II = Morinaga Institute ELISA Kit II

NL = nutriLinia® Allergen-ELISA

RS-F = Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

For the spiking level sample 64% (9) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 57% (8) of the recovery rates were within the range of acceptance.

To calculate the nominal values for sample A:

Besides the addition of skimmed milk powder (see page 5), sample A contains total milk protein or casein from the matrix instant sauce powder. For the calculation of the recovery rates of the participants' results, therefore, the average contents of total milk protein and casein from sample B were taken into account. The results for sample B were set as 100% content in the matrix, the added content to sample A then corresponds to about another 41%. This results in the sum of the following nominal values which were used for the calculation of the recovery rate for sample A: total milk protein 44.0 mg/kg and casein 34.4 mg/kg.

4.1.3 PCR Results: Milk

No PCR results were submitted for the parameter milk.

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 consensus value		
14	positive	16,5	negative		2/2 (100%)	AT	Result converted °
15	positive	12,0	negative	<0,9	2/2 (100%)	BC	
7	positive	57,9	negative	0	2/2 (100%)	BF	
8	positive	34,3	negative	<0,31	2/2 (100%)	MI-II	
18	positive	33,0	negative	<0,31	2/2 (100%)	MI-II	
3	positive	26,5	negative		2/2 (100%)	RS-F	
6	positive	> 20	negative	< 2,5	2/2 (100%)	RS-F	
9a	positive	11,6	negative	<2,5	2/2 (100%)	RS-F	Result converted °
10	positive	>20	negative	<2,5	2/2 (100%)	RS-F	
11	positive	32,0	negative	0	2/2 (100%)	RS-F	
12	positive	34,0	negative	< 0,31	2/2 (100%)	RS-F	
13	positive	82,7	negative	<2,5	2/2 (100%)	RS-F	
21	negative	< 2,5	positive	142	0/2 (0%)	RS-F	
24	positive	33,0	negative	<2,5	2/2 (100%)	RS-F	
9b	positive	21,1	negative	<1,8	2/2 (100%)	VT	Result converted °

° calculation see p. 19

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

Methods:

AT = AlerTox ELISA, Biomedal
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus values are in qualitative agreement with the spiking of sample A.
One participant obtained a positive result for sample B and a negative result for sample A.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Soy Protein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
14	16,5	-1,4	AT	Result converted °
15	12,0	-1,9	BC	
7	57,9	2,9	BF	
8	34,3	0,4	MI-II	
18	33,0	0,3	MI-II	
3	26,5	-0,4	RS-F	
6	> 20		RS-F	
9a	11,6	-2,0	RS-F	Result converted °
10	> 20		RS-F	
11	32,0	0,2	RS-F	
12	34,0	0,4	RS-F	
13	82,7	5,6	RS-F	
21	< 2,5		RS-F	
24	33,0	0,3	RS-F	
9b	21,1	-1,0	VT	Result converted °

° calculation see p. 19

Methods:

AT = AlerTox ELISA, Biomedal
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

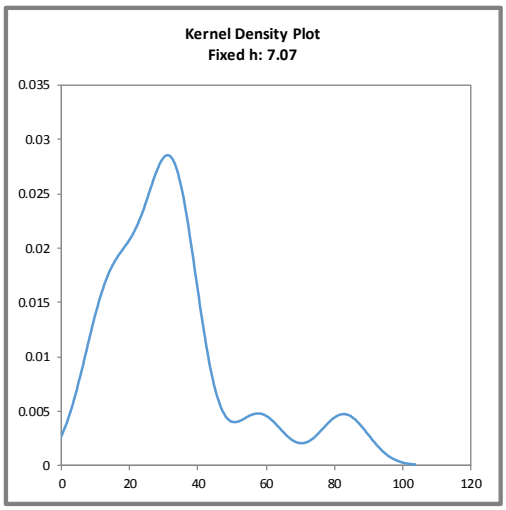


Abb. / Fig. 24:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 symmetrical distribution of results with a shoulder at approx. 12 mg/kg and two smaller peaks at approx. 60 mg/kg and 85 mg/kg due to single results.

Characteristics: Quantitative evaluation ELISA Soy Protein

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	12
Number of outliers	1
Mean	32,9
Median	32,5
Robust Mean (X)	**30,1**
Robust standard deviation (S*)	**15,7**
Target range:	
Target standard deviation $\sigma_{pt'}$	**9,43**
lower limit of target range	**11,3**
upper limit of target range	**49,0**
Quotient S/$\sigma_{pt'}$*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*5,67*
Results in the target range	*10*
Percent in the target range	*83*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences.

Due to the relatively broad distribution of results of the different methods the evaluation was done by z'-score considering the standard uncertainty. The quotient S/$\sigma_{pt'}$ was then 1,7.The robust standard deviations are in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

Due to the small number and distribution of the results, no separate evaluation was made for the method RS-F.

The robust mean of the evaluation of all results was 81% of the spiking level of soy protein to sample A and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soyprotein" p.58).

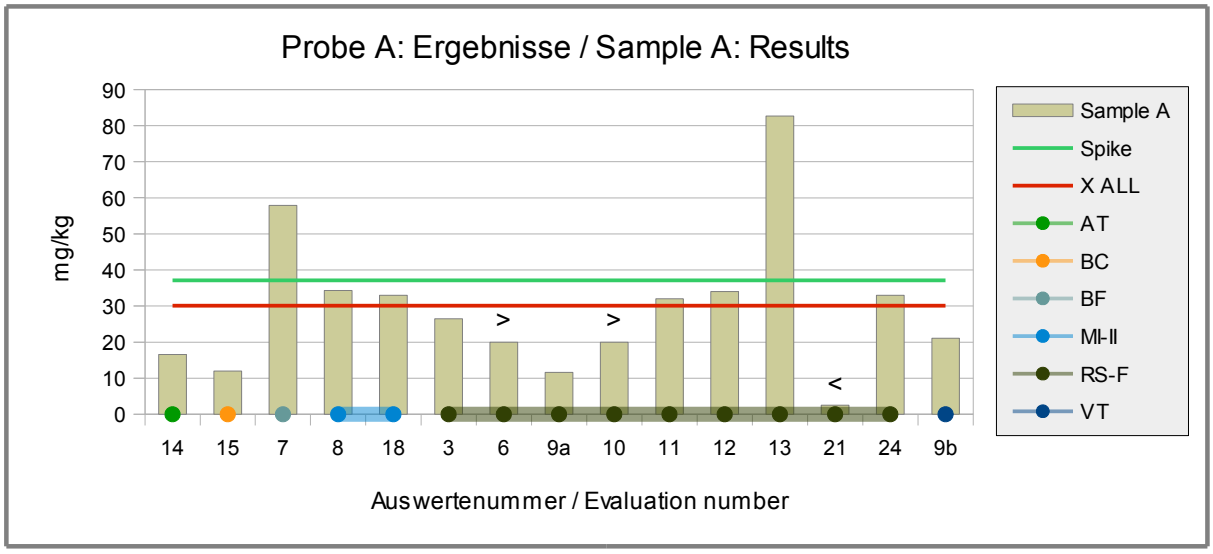


Abb./Fig. 25: ELISA Results soya (as soy protein)
green line = Spiking level
red line = Assigned value robust mean of all results
round symbols = Applied methods (see legend)

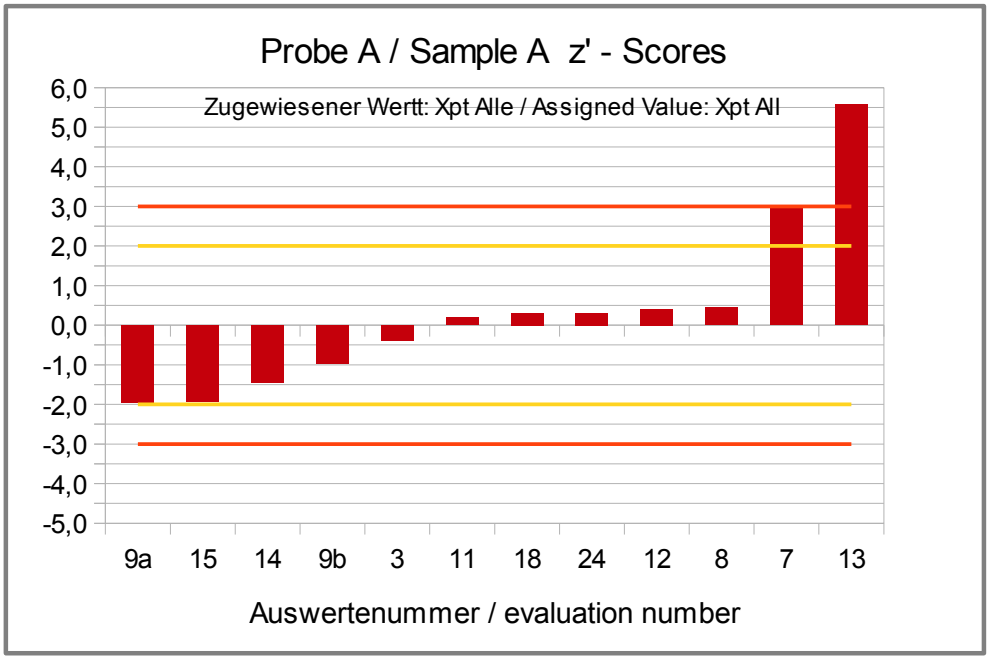


Abb./Fig. 26:
z'-Scores (ELISA Results soy protein)
Assigned value robust mean of all results

Quantitative Valuation of results: Spiking level sample

Evaluation number	Soy Protein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
14	30,0	-0,9	AT	Result converted °
15	15,7	-1,8	BC	
7	79,5	2,2	BF	
8	40,9	-0,2	MI-II	
18	28,0	-1,0	MI-II	
3			RS-F	
6			RS-F	
9a	13,8	-1,9	RS-F	Result converted °
10	>20		RS-F	
11	36,0	-0,5	RS-F	
12	47,0	0,1	RS-F	
13	100	3,4	RS-F	
21	155	6,8	RS-F	
24	38,0	-0,4	RS-F	
9b	21,4	-1,4	VT	Result converted °

° calculation see p. 19

Methods:

AT = AlerTox ELISA, Biomedal
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

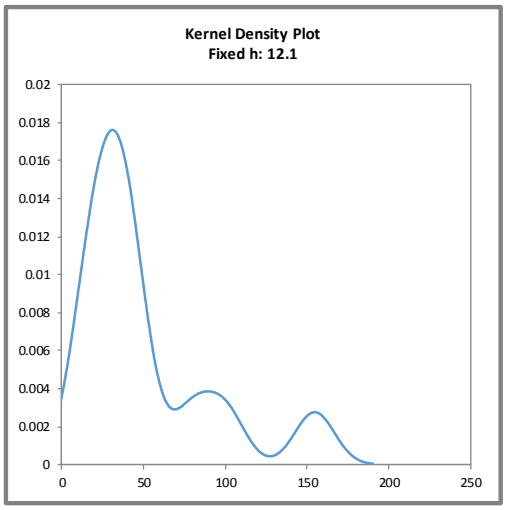


Abb. / Fig. 27:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a small peak at approx. 90 mg/kg and a small peak at approx. 155 mg/kg due to one outlier (method RS-F).

Characteristics: Quantitative evaluation ELISA Soya (as soy protein)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	12
Number of outliers	1
Mean	50,4
Median	37,0
Robust Mean (X_{pt})	**44,7**
Robust standard deviation (S*)	**32,1**
Target range:	
Target standard deviation $\sigma_{pt'}$	**16,1**
lower limit of target range	**12,5**
upper limit of target range	**76,8**
Quotient S/$\sigma_{pt'}$*	*2,0*
Standard uncertainty $U_{(Xpt)}$	*11,6*
Results in the target range	*9*
Percent in the target range	*75*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences.

The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} of > 2. Therefore the evaluation of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then 2,0. The robust standard deviations are in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

Due to the small number and distribution of the results, no separate evaluation was made for the method RS-F.

The robust mean of the evaluation of all results was 120% of the spiking level of soy protein to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of soy protein" p.58).

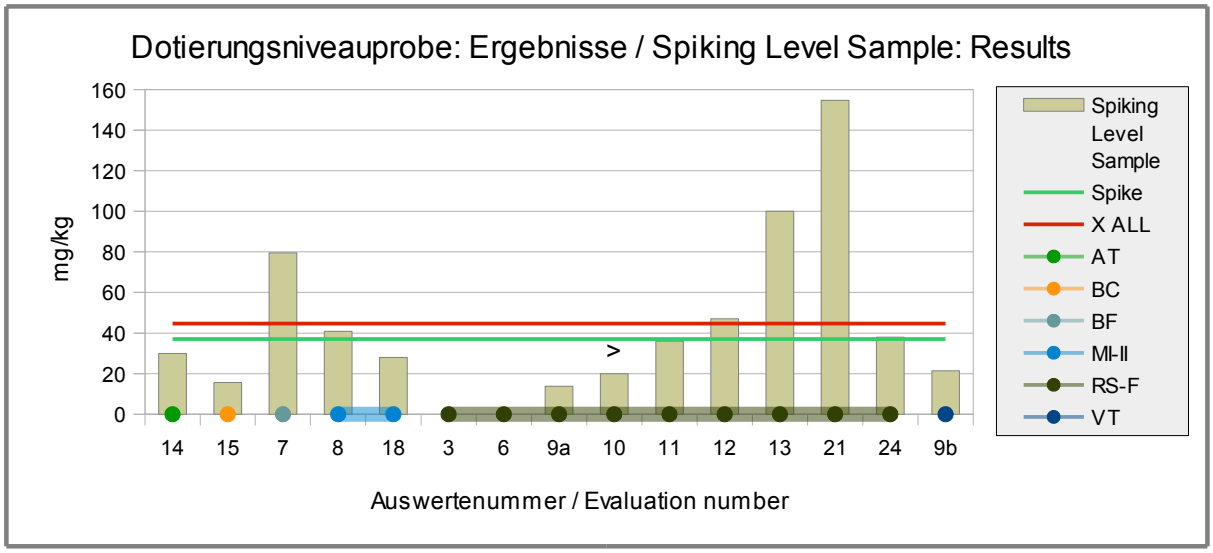


Abb./Fig. 28: ELISA Results soy (as soy protein)
green line = Spiking level
red line = Assigned value robust mean of all results
round symbols = Applied methods (see legend)

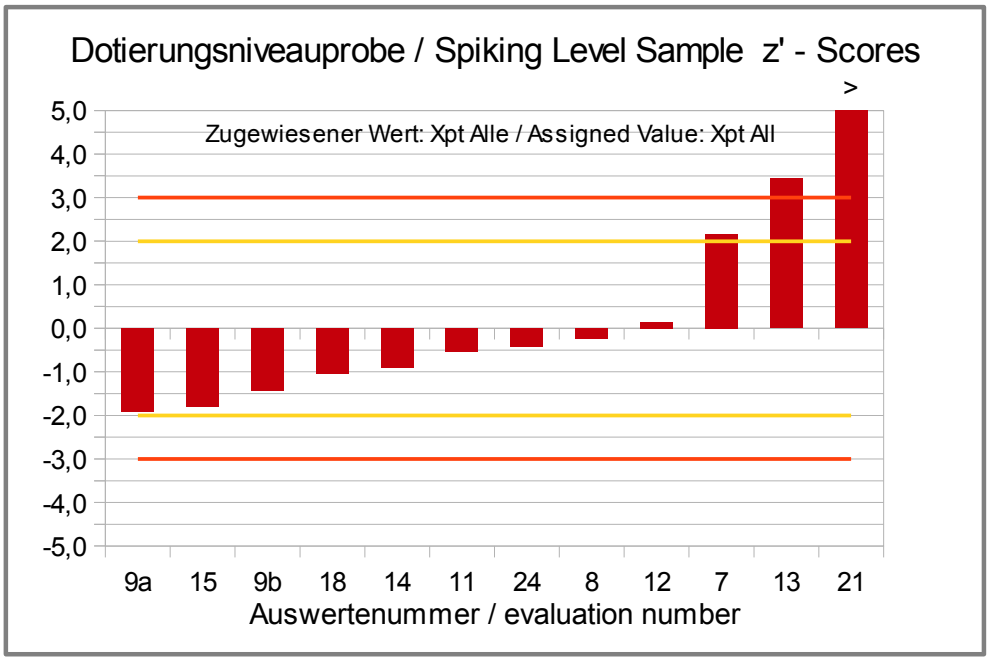


Abb./Fig. 29:
z'-Scores (ELISA Results soy protein)
Assigned value robust mean of all results

Recovery Rates ELISA for Soy Protein:
Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
14	30,0	**81**	16,5	45	AT	Result converted °
15	15,7	42	12,0	32	BC	
7	79,5	214	57,9	156	BF	
8	40,9	**110**	34,3	**92**	MI-II	
18	28,0	**75**	33,0	**89**	MI-II	
3			26,5	**71**	RS-F	
6			> 20		RS-F	
9a	13,8	37	11,6	31	RS-F	Result converted °
10	>20		>20		RS-F	
11	36,0	**97**	32,0	**86**	RS-F	
12	47,0	**127**	34,0	**92**	RS-F	
13	100	270	82,7	223	RS-F	
21	155	417	<2,5		RS-F	
24	38,0	**102**	33,0	**89**	RS-F	
9b	21,4	**58**	21,1	**57**	VT	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**7**	Number in RA	**7**
Percent in RA	**58**	Percent in RA	**58**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AT = AlerTox ELISA, Biomedal
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
58% (7) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150% with the spiked food matrix sample A and the spiking level sample.

4.2.2 ELISA Results: Soya (as Soy Trypsin Inhibitor)

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	0,700	negative	<LOD	2/2 (100%)	AQ	
19	positive	0,700	negative	<LOD	2/2 (100%)	AQ	
20	positive	0,780	negative	< LOD	2/2 (100%)	AQ	
16	positive	0,711	negative	< 0,04	2/2 (100%)	EF	
23	positive	0,830	negative	< 0,016	2/2 (100%)	EF	
5	positive	0,890	negative	<LOQ	2/2 (100%)	IL	

	Sample A		Sample B	
Number positive	6		0	
Number negative	0		6	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:
AQ = AgraQuant, RomerLabs
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Soy trypsin inhibitor	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
1	0,700	-0,4	AQ	
19	0,700	-0,4	AQ	
20	0,780	0,1	AQ	
16	0,711	-0,3	EF	
23	0,830	0,3	EF	
5	0,890	0,6	IL	

Methods:

AQ = AgraQuant, RomerLabs

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

Comments:

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

Characteristics: Quantitative evaluation ELISA Soya

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	0,769
Median	0,746
Robust Mean (X_{pt})	**0,769**
Robust standard deviation (S*)	**0,0898**
Target range:	
Target standard deviation σ_{pt}	**0,192**
lower limit of target range	**0,384**
upper limit of target range	**1,15**
Quotient S/σ_{pt}*	*0,47*
Standard uncertainty $U_{(Xpt)}$	*0,0458*
Results in the target range	*6*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a low variability of results with a quotient S/σ_{pt} below 1,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust mean of the evaluation of all results was 14% of the spiking level of soy trypsin inhibitor to sample A and thus, subject to the note below, below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soy trypsin inhibitor" p.66).

Note: According to the test kit instructions, the conversion of the soy trypsin inhibitor results requires a conversion factor of 42 for un-toasted soyflour and a conversion factor of 470 for toasted soyflour. For the soyflours 32,3 mg/kg or 361 mg/kg are obtained with recovery rates of 33% and 370%, respectively. The soyflour used in this PT is a mixture of toasted soybeans that has a residual activity of the soy trypsin inhibitor.

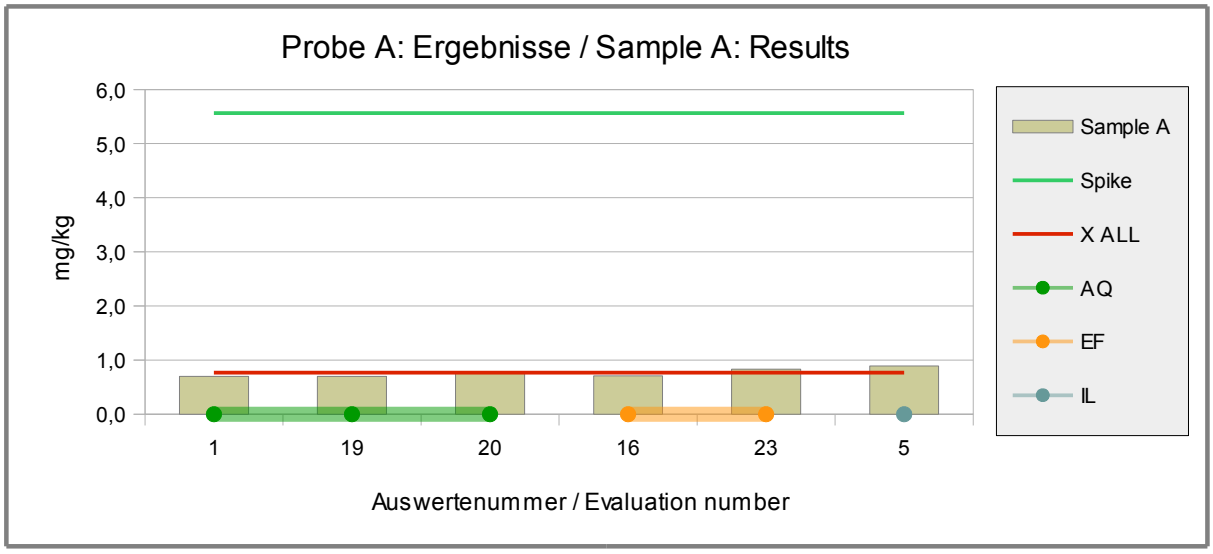


Abb./Fig. 30: ELISA Results soya (as soy trypsin inhibitor)
green line = Spiking level
red line = Assigned value robust mean of all results
round symbols = Applied methods (see legend)

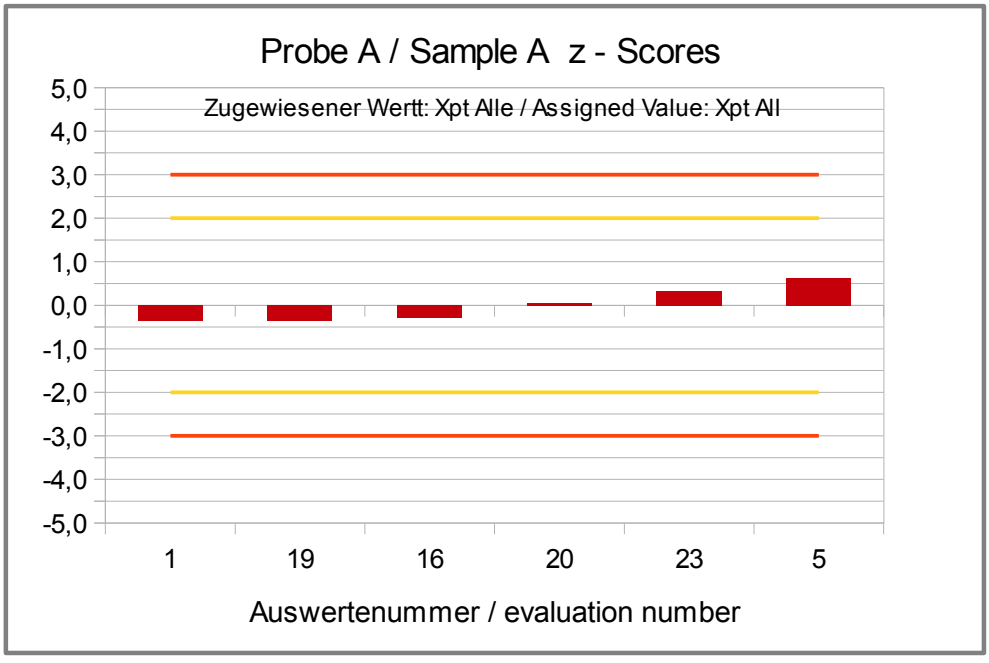


Abb./Fig. 31:
z-Scores (ELISA Results as soy trypsin inhibitor)
Assigned value robust mean of all results

Quantitative Valuation of results: Spiking level sample

Evaluation number	Soy Trypsin Inhibitor	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
1	0,800	-0,7	AQ	
19	0,870	-0,4	AQ	
20	1,18	0,9	AQ	
16	1,12	0,6	EF	
23	0,850	-0,5	EF	
5	1,00	0,1	IL	

Methods:
Methoden:
AQ = AgraQuant, RomerLabs
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab

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

Characteristics: Quantitative evaluation ELISA Soya (as soy trypsin inhibitor)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	0,970
Median	0,935
Robust Mean (X_{pt})	**0,970**
Robust standard deviation (S*)	**0,176**
Target range:	
Target standard deviation σ_{pt}	**0,243**
lower limit of target range	**0,485**
upper limit of target range	**1,46**
Quotient S/σ_{pt}*	*0,73*
Standard uncertainty $U_{(Xpt)}$	*0,0899*
Results in the target range	*6*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a low variability of results with a quotient S/σ_{pt} below 1,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust mean of the evaluation of all results was 17% of the spiking level of soy trypsin inhibitor to the spiking level sample and thus, subject to the note below, below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soy trypsin inhibitor" p.66).

Note: According to the test kit instructions, the conversion of the soy trypsin inhibitor results requires a conversion factor of 42 for untoasted soyflour and a conversion factor of 470 for toasted soyflour. For soyflour 40,7 mg/kg or 456 mg/kg are obtained with recovery rates of 42% and 654%, respectively. The soyflour used in this PT is a mixture of toasted soybeans that has a residual activity of the soy trypsin inhibitor.

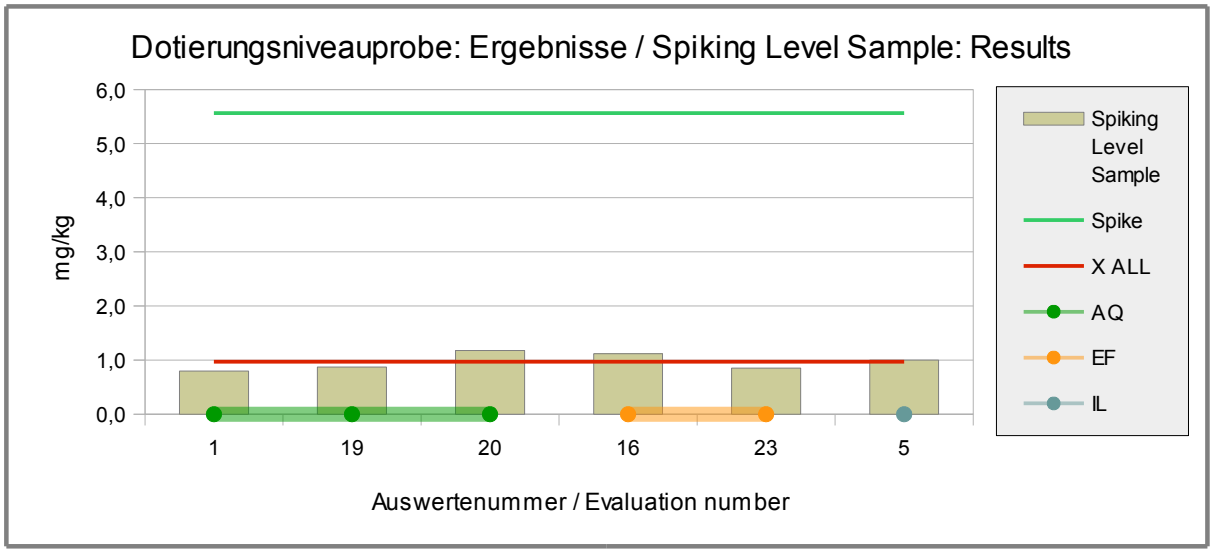


Abb./Fig. 32: ELISA Results soy (as soy trypsin inhibitor)
green line = Spiking level
red line = Assigned value robust mean of all results
round symbols = Applied methods (see legend)

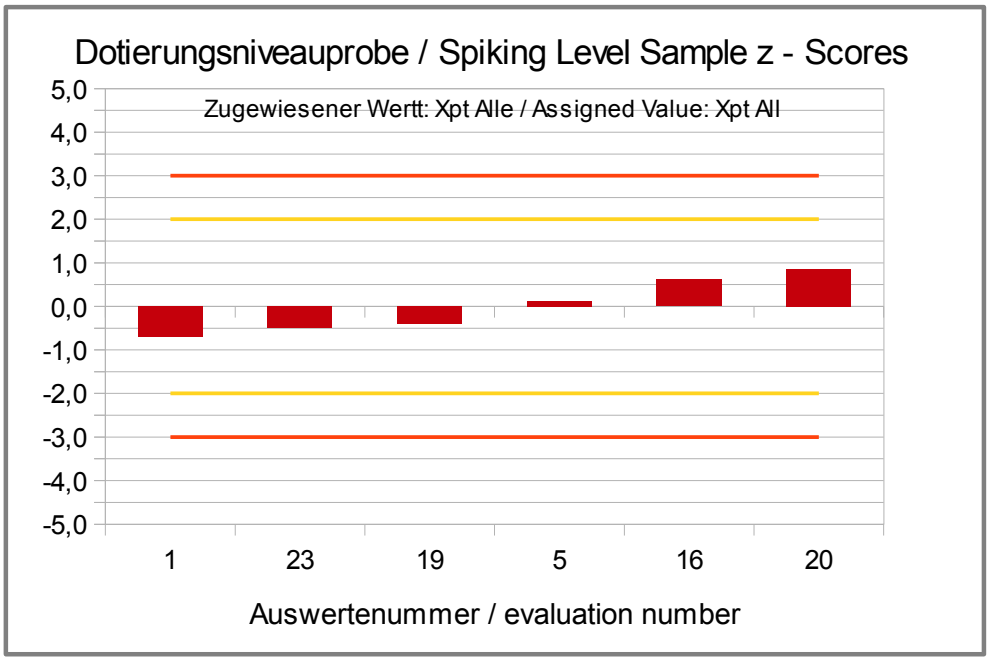


Abb./Fig. 33:
z-Scores (ELISA Results as soy trypsin inhibitor)
Assigned value robust mean of all results

Recovery Rates ELISA for Soy Trypsin Inhibitor: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	0,800	14	0,700	13	AQ	
19	0,870	16	0,700	13	AQ	
20	1,18	21	0,780	14	AQ	
16	1,12	20	0,711	13	EF	
23	0,850	15	0,830	15	EF	
5	1,00	18	0,890	16	IL	

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

Methods:
AQ = AgraQuant, RomerLabs
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab

* Recovery rate 100% relative size: soy trypsin inhibitor, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Comments:

For the indication of results as soy trypsin inhibitor, all recovery rates for both the spiking level sample and the spiked food matrix sample A are below the range of the AOAC-recommendation of 50-150%. According to the test kit instructions of the ELISA methods AQ, EF and IL, for the conversion of soy trypsin inhibitor results a conversion factor of 42 for untoasted soyflour and a conversion factor of 470 for toasted soyflour should be applied. Depending on the factor used for the spiking level sample mean recovery rates of 42% and 465% are obtained and for the food matrix sample A of 33% and 370%, respectively. The soyflour used in this PT is a mixture of toasted soybeans that has a residual activity of the soy trypsin inhibitor.

4.2.3 PCR Results: Soya

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		
4	positive	85,0	negative		2/2 (100%)	ASU	
17	positive	70,0	negative		2/2 (100%)	ASU	
2	positive		negative		2/2 (100%)	FP	given as soya DNA
22	positive		negative		2/2 (100%)	GI	
9	positive	61,7	negative	< 1	2/2 (100%)	SFA	
16	positive		negative		2/2 (100%)	SFA	
10	positive		negative		2/2 (100%)	SFA-ID	
6	positive		negative		2/2 (100%)	div	
12	positive	33,0	negative	< 10	2/2 (100%)	div	given as soya DNA
18a	positive		negative		2/2 (100%)	div	
18b	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	11		0	
Number negative	0		11	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative Valuation PCR: Sample A

No quantitative evaluation was done, because there were too few individual results.

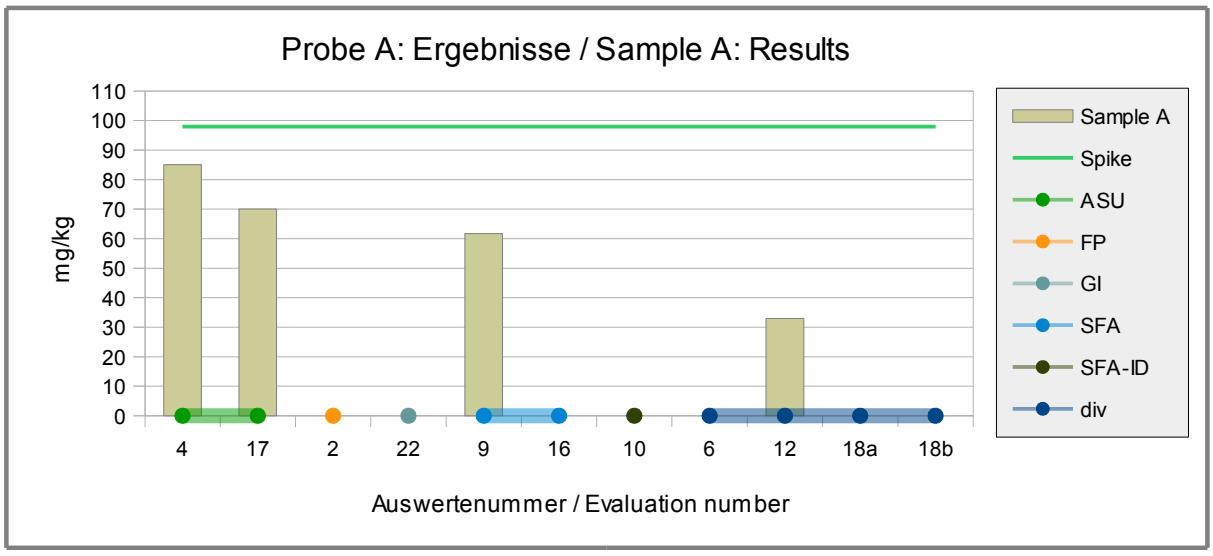


Abb./Fig. 34: PCR Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation PCR: Spiking Level Sample

No quantitative evaluation was done, because there were to few quantitative results.

Evaluation number	Soya	Soya	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive	42,0		ASU	
17	positive	92,0		ASU	
2	positive	277		FP	given as soya DNA
22				GI	
9	positive	62,1		SFA	
16	positive			SFA	
10	positive			SFA-ID	
6	positive			div	
12	positive	53,0		div	given as soya DNA
18a	positive			div	
18b	positive			div	

Number positive	10	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:

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

Comments:
For the spiking level sample there were 100% positive results.

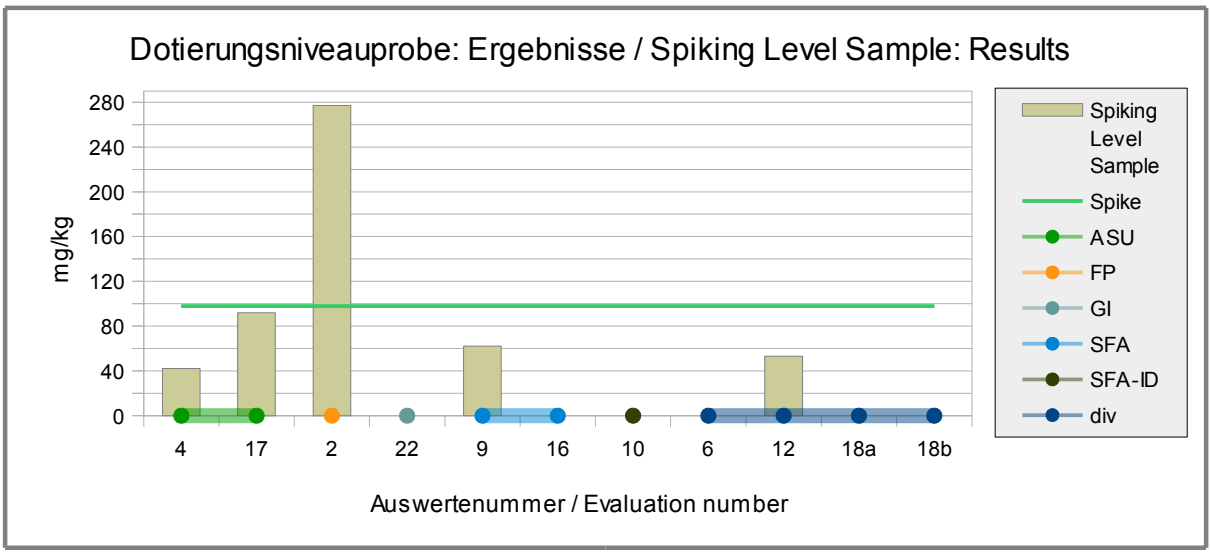


Abb./Fig. 35: PCR Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Soya:
Spiking Material Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4	42,0	43	85,0	**87**	ASU	
17	92,0	**94**	70,0	**71**	ASU	
2	277				FP	Result given as soy DNA (RR as soyflour would be 280%)
22					GI	
9	62,1	**63**	61,7	**63**	SFA	
16					SFA	
10					SFA-ID	
6					div	
12	53,0		33,0		div	Result given as soy DNA (RR as soyflour would be 54% and 34%)
18a					div	
18b					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**2**	Number in RA	**3**
Percent in RA	**67**	Percent in RA	**100**

* Recovery rate 100% relative size: soybean/ soyflour, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:
ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
GI = GEN-IAL First Allergen
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
For the spiking level sample, the recovery rates by PCR were between 43% and 94%, of which 2 were in the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A, 3 of the recovery rates were in this range of acceptance.

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: Casein

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
AQ	1	25.02.19	positive	13,3	positive	8,3	positive	22,5	0,04	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	4	26.02.19	positive	78,1	positive	51,8	positive	81	1,9	7	50	skimmed milk powder	AgraQuant Casein COKAL 1200, RomerLabs
AQ	6	06.03.19	positive	12,3	positive	8,71	positive	16	0,2	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	15	21.02.19	-	10,2	-	6,6	-	11,6	0,2	0,2	50	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	19	06.02.19	positive	18,8	positive	14,6	positive	23,4	0,04	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
BF	7	05.03.19	positive	20,6	positive	17,2	positive	22,8	0,38	3		Milk proteins, total	MonoTrace Milk (Casein) ELISA kit, BioFront Technologies
EF	16		-	18,5	-	14	-	22	0,04	0,2	30	Casein	SENSISpec ELISA Casein - Eurofins
IL	5	07.03.19	-	14,85	-	12,95	-	26,35	0,04	0,2		Casein	Immunolab Casein ELISA
MI	8	26.02.19	positive	59,4	positive	37,2	positive	23,5		0,31	45,1	Milk proteins, total	Morinaga Casein ELISA Kit
MI-II	18	31.01.19	positive	42	positive	35	positive	17	0,31	0,31		Milk proteins, total	Morinaga Casein ELISA Kit II M2113
NL	12	20.02.19	positive	19	positive	13	positive	21	0,05	0,2	30	Casein	nutriLinia® Allergen-ELISA Casein-E NC-6031
RS-F	9	11.02.19	positive	43,88	positive	32,53	positive	22,45	2,5	2,5	15,93	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	11	11-12 February	positive	14	positive	7,3	positive	4,4	0,12	0,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	24	03.06.19	positive	35	positive	25	positive	18		2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Casein:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	1	Casein	Extraction buffer/ 15 minutes / 60C	yes	
AQ	4		according to manufacturer's instructions	yes	conversion factor 3,6
AQ	6			yes	
AQ	15	polyclonal	0.5g sample PBS Buffer extraction at 60°C		
AQ	19	Casein	aqueous buffer / 15 minutes / 60°C	yes	
BF	7	Monoclonal antibody-based kit	1:10 extraction ratio/10 minutes/60C	no	
EF	16			yes	
IL	5			No	
MI	8	Casein	Morinaga Casein ELISA Kit II	Yes	extraction 100°C 10 min
MI-II	18	recognizes cow's milk casein	according to manufacturer's instructions	yes	Casein mg/kg Sample A: 34, Sample B: 28, spiking level sample: 14
NL	12		according to the manual	yes	
RS-F	9	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	11			yes	
RS-F	24				

5.1.2 ELISA: Milk protein

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
AQ	4	28.02.19	positive	58,3	positive	33,5	positive	61	2,5		50	skimmed milk powder	AgraQuant ELISA Milk COKAL2448, RomerLabs
AQ	12a	20.02.19	positive	27	positive	16	positive	27	0,05	0,4	30	Milk proteins, total	AgraQuant ELISA Milk COKAL2448 von RomerLabs
AQ	19	05.02.19	positive	16,9	positive	12,9	positive	22	0,05	0,4	35	Milk proteins, total	AgraQuant ELISA Milk COKAL2448, RomerLabs
AQ	20	30.01.19	positive	20	positive	15,1	positive	17,8	0,05	0,4	35	Milk proteins, total	AgraQuant ELISA Milk COKAL2448, RomerLabs
EF	16		-	21,7	-	10,8	-	35,9	0,05	0,4	30	Milk proteins, total	SENSISpec ELISA Total Milk - Eurofins
EF	23	04.02.19	positive	25	positive	15	positive	25	0.05	0.4		Casein + BLG	SensiSpec Milk ELISA
MI-II	8	26.02.19	positive	54,5	positive	26,6	positive	16,5		0,31	50,6	Milk proteins, total	Morinaga BLG ELISA Kit II
NL	12b	25.02.19	positive	26	positive	16	positive	36	0,05	0,5	30	Milk proteins, total	nutriLinia® Allergen-ELISA Milch-E / Milk-E NC-6033
RS-F	5	22.02.19		55,7	-	37,94	-	16,78	0,7	2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	9	11.02.19	positive	67,86	positive	42,25	positive	20,67	2,5	2,5	24,55	Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	11	11-12 February	positive	109	positive	60	positive	57	0,7	2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	21	18.02.19	-	47,9	-	32,5	-	11,9	0,7	2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
VT	3	07.03.19	positive	>25.0	positive	>25.0	-		0.24	43587	25	Milk proteins, total	Veratox Total Milk Allergen, Neogen
VT	6	06.02.19	positive	101	positive	58	positive	21,6	2,5	2,5		skimmed milk powder	Veratox Total Milk Allergen, Neogen
VT	17	08.02.19	positive	117	positive	98	positive	48	2,5	5	50	skimmed milk powder	Veratox Total Milk Allergen, Neogen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	4		according to manufacturer's instructions	no	conversion factor 2,7
AQ	12a		according to manual	no	
AQ	19	Milk proteins, total	aqueous buffer / 15 minutes / 60°C	yes	
AQ	20	Milk proteins, total	aqueous buffer / 15 minutes / 60°C	no	
EF	16			yes	sum of casein and BLG
EF	23	BLG, Casein			
MI-II	8	β-Lactoglobulin		Yes	extraction 100°C 10 min
NL	12b		according to manual	yes	
RS-F	5			No	No dillution
RS-F	9	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	11			yes	
RS-F	21	unknown	As Per Kit Instructions	yes	unknown
VT	3		5g of sample / 125ml extraction solution from kit / 15 min / 60 °C	yes	
VT	6			yes	Screening method (single determination)
VT	17				reduced weight of 1g

5.1.3 ELISA: Soy protein

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
AT	14	28.02.19	positive	43,75	negative		positive	79,24	7,6	23,75		Soybean	AlerTox Soy (STI) ELISA, Biomedal
BC	15	21.02.19	-	12	-	<0.9	-	15,7	0,9	0,9	50	Soyprotein	BioCheck ELISA Soya-Check
BF	7	05.03.19	positive	57,9	negative	0	positive	79,5	0,16	1		Soyprotein	MonoTrace Soy ELISA kit, BioFront Technologies
MI-II	8	26.02.19	positive	34,3	negative	<0.31	negative	40,9		0,31	45,2	Soyprotein	Morinaga Soya ELISA Kit II
MI-II	18	31.01.19	positive	33	negative	<0,31	positive	28	0,31	0,31		Soyprotein	Morinaga Soya ELISA Kit II
RS-F	3	07.03.19	positive	26.50	negative	43587	-		0.24	43587	25	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	6	05.02.19	positive	> 20	negative	< 2,5	-		2,5			Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	9a	21.02.19	positive	30,75	negative	<2.5	positive	36,55	2,5	2,5	29,35	Soybean	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	10	04.02.19	positive	>20	negative	<2,5	positive	>20	1	2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	11	25.02-01.03	positive	32	negative	0	positive	36	0,24	2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	12	21.02.19	positive	34	negative	< 0,31	positive	47	0,31	2,5	30	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	13	05.03.19	positive	82,7	negative	<2,5	positive	100	0,24	2,5	63,1	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	21	15.02.19	-	< 2.5	-	142,4	-	154,7	0,24	2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	24	03.07.19	positive	33	negative	<2,5	positive	38		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
VT	9b	13.02.19	positive	44,8	negative	<2.5	positive	45,6	2,5	2,5	23,11	Soyflour	Veratox Soy Allergen, Neogen

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Soy protein:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AT	14				
BC	15	polyclonal	0.5g sample PBS Buffer extraction at 60°C		
BF	7	Monoclonal antibody-based kit	1:20 extraction ratio/10 minutes/Boiling	no	
MI-II	8	Beta-conglycinin		Yes	extraction 100°C 10 min
MI-II	18	recognizes soyprotein	according to manufacturer's instructions	Yes	
RS-F	3		1 g of sample / 2,5 ml Extractor 3 + 17.5 ml Extraction buffer (60 °C) / 10 min. / 100 °C	yes	
RS-F	6			Yes	
RS-F	9a	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	10				
RS-F	11			Yes	
RS-F	12		according to manual	Yes	
RS-F	13		Sample preparation and test procedure is carried out as described in the test kit	Yes	
RS-F	21	unknow n	As Per Kit Instructions	Yes	Cross-reactivity w ith legumes, peas and peanuts
RS-F	24				
VT	9b	As Per Kit Instructions	As Per Kit Instructions	Yes	

5.1.4 ELISA: Soya Trypsin Inhibitor

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	Test-Kit + Manufacturer
AQ	1	19.02.19	positive	0,7	negative	<LOD	positive	0,8	0,016	0,04	35	Soy Trypsin Inhibitor	AgraQuant ELISA Soy COKAL0448, RomerLabs
AQ	19	08.02.19	positive	0,7	negative	<LOD	positive	0,87	0,016	0,04	35	Soy Trypsin Inhibitor	AgraQuant ELISA Soy COKAL0448, RomerLabs
AQ	20	07.02.19	positive	0,78	negative	< LOD	positive	1,18	0,016	0,04	35	soy trypsin inhibitor	AgraQuant ELISA Soy COKAL0448, RomerLabs
EF	16		-	0,711	-	< 0.04	-	1,12	0,016	0,04	30	Soya trypsin inhibitor	SENSISpec ELISA Soya - Eurofins
EF	23	04.02.19	positive	830 ppb	negative	< 16 ppb	positive	850 ppb	16 ppb	40 ppb		Soya trypsin inhibitor	SensiSpec Soy ELISA
IL	5	19.02.19	-	37,2	negative	<LOQ	-	41,82	0,7	1,7		Soyflour	Immunolab Soy ELISA
IL	5	19.02.19	-	890 ppb	negative	<LOQ	-	1000 ppb				Soy Trypsin Inhibitor	Immunolab Soy ELISA

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	1	STI - soy trypsin inhibitor	Extraction buffer/ 15 minutes / 60C	yes'	
AQ	19	STI- soy trypsin inhibitor	aqueous buffer / 15 minutes / 60°C	yes	
AQ	20	STI - soy trypsin inhibitor	aqueous buffer / 15 minutes / 60°C	no	
EF	16			yes	
EF	23	STI			
IL	5			No	Dillution 1:5
IL	5			No	Dillution 1:5

5.1.5 PCR: Soya

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	PCR Test-Kit + Manufacturer
ASU	4	21.02.19	positive	85	negative		positive	42	10	20	50	soyflour	ASU §64 Methode/method
ASU	17	11.02.19	positive	70	negative		positive	92	5	10	50	soyflour	ASU §64 Methode/method
FP	2		positive		negative		positive	277,08				Soy-DNA	foodproof Detection Kit, BIOTECON Diagnostics
GI	22		positive		negative		-		10	< 100 mg/kg			GEN-IAL First Allergen, Romerlabs Deutschland
SFA	9	05.03.19	positive	61,74	negative	<1	positive	62,14	1	1	39,2	Soybean	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		positive		negative		positive		0,4			Soy-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	10	06.02.19	positive		negative		positive		0,4			Soy-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	6	18.02.19	positive		negative		positive					Soy-DNA	DIN EN ISO 21570, Anhang C2, Ausgabe August 2013, modifiziert
div	12	01.03.19	positive	33	negative	< 10	positive	53	10 copies	30 copies	40	Soy-DNA	
div	18a	01.02.19	positive		negative		positive		40			Soy-DNA	internal method
div	18b	01.02.19	positive		negative		positive		4-10			Soy-DNA	internal method

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4	Soy lectin gene	Automated extraction (Maxwell RSC), 45 cycles	Yes	
ASU	17	Lectin gene, 81 bp	Extraction: CTAB precipitation method, s. e.g. ASU L 18.00-22 Determination: ASU L 08.00-59 : 2013-01	Yes	Calibration/Quantification with matrix standards, spiked material: soyflour.
FP	2		real time PCR	no	
GI	22	soy lectin		Yes	100 mg/Kg ist LOQ
SFA	9	As Per Kit Instructions	CONGEN SureFood Advanced Prep Kit	Yes	Kit S6301
SFA	16			yes	
SFA-ID	10				
div	6	74 bp Soy lectin 1 Gen	MericonFood Kit (Qiagen)	Yes	
div	12	Lectin		Yes, house method	
div	18a		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime PCR 45 cycles	Yes	
div	18b		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime PCR 45 cycles	Yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before botteling

Microtracer Homogeneity Test
DLA 01-2019 Sample A

Weight whole sample	4,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	39	15,4
2	5,06	44	17,4
3	4,98	45	18,1
4	5,00	50	20,0
5	5,01	43	17,2
6	5,03	46	18,3
7	5,00	40	16,0
8	5,01	52	20,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	44,9	Particles
Standard deviation	4,57	Particles
χ^2 (CHI-Quadrat)	3,25	
Probability	**86**	%
Recovery rate	80	%

Normal distribution		
Number of samples	8	
Mean	17,9	mg/kg
Standard deviation	1,82	mg/kg
rel. Standard deviaton	10,2	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,0**	
Recovery rate	80	%

Microtracer Homogeneity Test
DLA 01-2019 Spiking Level Sample

Weight whole sample	1,44	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	57	22,8
2	5,00	54	21,6
3	4,97	59	23,7
4	5,09	63	24,8
5	5,06	58	22,9
6	5,02	66	26,3
7	5,06	67	26,5
8	5,09	49	19,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,1	Particles
Standard deviation	6,08	Particles
χ^2 (CHI-Quadrat)	4,38	
Probability	**74**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	23,5	mg/kg
Standard deviation	2,41	mg/kg
rel. Standard deviaton	10,3	%
Horwitz standard deviation	9,9	%
HorRat-value	**1,0**	
Recovery rate	105	%

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 01-2019***
PT name	***Allergens I: Milk (Casein) and Soya in Sauce Powder***
Sample matrix (processing)	***Samples A + B:*** *Instant Sauce Powder/ ingredients: maltodextrin, starch, modified starch, iodised salt, wheat flour, sugar, flavorings, yeast extract, onions, spices, caramel, tomato powder, corn oil, malt extract, acidity regulators: sodium diacetate, calcium lactate, acidifier: citric acid. Lactic acid, thyme. , 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: room temperature (long term cooled 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Skimmed milk powder (milk protein, casein, DNA), Soyflour (soy protein, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest March 08th 2019***
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	*Dr. Matthias Besler-Scharf*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		USA
		CANADA
		ITALY
		Germany
		Germany
		Germany
		SPAIN
		Germany
		ITALY
		Germany
		Germany
		Germany
		SWITZERLAND
		SPAIN
		Germany
		Germany
		GREAT BRITAIN
		AUSTRIA
		AUSTRIA
		USA
		CROATIA
		SLOVAKIA
		GREECE

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

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

7. Index of references

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20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods -

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