Evaluation Report

proficiency test

DLA ptAL01 (2021)

Allergens I:

Milk (Casein) and Soya

in Sauce Powder

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General Information on the proficiency test (PT)

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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.
Akkreditierung *Accreditation* ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-EP-21534-01-00	nach / according to: ISO/IEC 17.043-2010 Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen Conformity Assessment - General Requirements for Proficiency Testing Die Akkreditierung gilt für den in der Urkundenanlage genannten Umfang. The accreditation is valid for the scope of the annex to the certificate of accreditation

Contents

1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a customary instant sauce powder. The basic composition of samples A and B was the same (see table 1). After crushing and sieving of the raw materials 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 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	**Probe A**	**Probe B**	**Dotierungs-niveauprobe**
Vegetable Sauce powder Ingredients: Starch (potato), salt, vegetables (onions, carrots, leeks, parsnips, potatoes, celery, garlic, parsley), corn starch, raw cane sugar, herbs, spices, sunflower oil, flavor enhancer (sodium gluatamate, disodium inosinate), anti-caking agent: silicon dioxide; natural fenugreek flavor	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***	 44,7 mg/kg 14,8 mg/kg 11,8 mg/kg 4,5 mg/kg	-	 55,2 mg/kg 18,2 mg/kg 14,6 mg/kg 5,5 mg/kg
Soya: soyflour-mixture, toasted (6 products from Asia, Europe, North Amerika) - as Soyflour* - *thereof 37,8 total protein*** - *thereof soy trypsin inhibitor****	 59,8 mg/kg 22,6 mg/kg 3,4 mg/kg	-	 62,8 mg/kg 23,7 mg/kg 3,6 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.*

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 91%. 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 0,89 and 0,78. 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 (exception: Morinaga ELISA II performed by DLA). The sample weights were made with a deviation of ± 10% from recommended sample weight of the test kit instructions and not communicated to the laboratories. After transmission of analysis results by the laboratories, the valid results were calculated on the basis of the exact weightings by DLA and the statistical calculation was carried out according to ISO 13528:2015 Annex B (possibly with Notes 1 and 2).

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

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

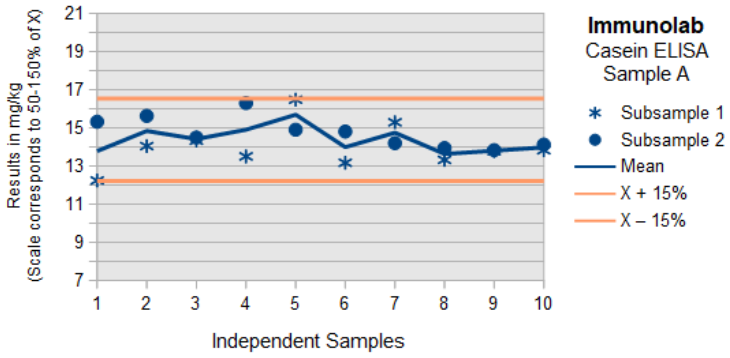
ELISA-Tests: Homogenität Milch (Casein) / Homogeneity Milk (Casein)

Immunolab Casein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Casein 14,4 ± 0,32 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	12,2	15,3	13,8
2	14,0	15,6	14,8
3	14,3	14,5	14,4
4	13,5	16,3	14,9
5	16,5	14,9	15,7
6	13,2	14,8	14,0
7	15,3	14,2	14,7
8	13,3	13,9	13,6
9	13,8	13,8	13,8
10	13,8	14,1	14,0

General average X	14,4	
SD of sample means Sx	0,66	4,6%
SD within-samples Sw	0,82	5,7%
SD between-samples Ss	0,32	2,2%

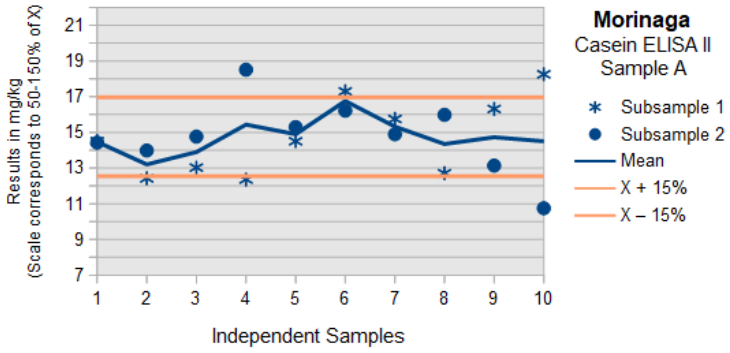


Morinaga Casein ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Whole milk protein 14,8 ± 0,96 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	14,5	14,4	14,5
2	12,4	14,0	13,2
3	13,0	14,8	13,9
4	12,4	18,5	15,4
5	14,5	15,3	14,9
6	17,3	16,2	16,8
7	15,7	14,9	15,3
8	12,7	16,0	14,3
9	16,3	13,1	14,7
10	18,3	10,8	14,5

General average X	14,8	
SD of sample means Sx	0,96	6,5%
SD within-samples Sw	1,75	11,9%
SD between-samples Ss	< 0,96	< 6,5%

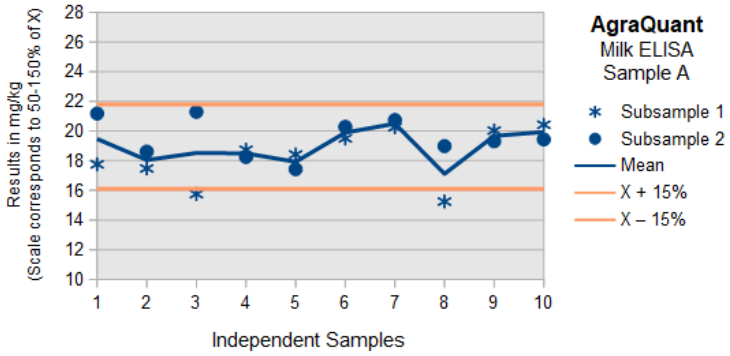


AgraQuant Milkprotein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Milkprotein 19,0 ± 0,65 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	17,8	21,2	19,5
2	17,5	18,6	18,0
3	15,7	21,3	18,5
4	18,7	18,3	18,5
5	18,4	17,4	17,9
6	19,5	20,3	19,9
7	20,3	20,7	20,5
8	15,2	19,0	17,1
9	20,0	19,3	19,7
10	20,4	19,4	19,9

General average X	19,0	
SD of sample means Sx	1,09	5,7%
SD within-samples Sw	1,24	6,5%
SD between-samples Ss	0,65	3,4%



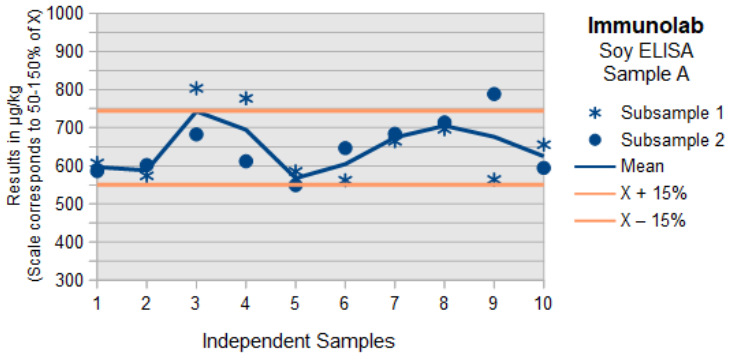
ELISA-Tests: Homogenität Soja / Homogeneity Soya

Immunolab Soya ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soy trypsin inhibitor 647 ± 46,0 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	607	586	597
2	574	602	588
3	803	682	743
4	777	612	694
5	586	549	567
6	562	646	604
7	664	684	674
8	696	714	705
9	564	788	676
10	656	595	625

General average X	647	
SD of sample means Sx	58,7	9,1%
SD within-samples Sw	51,5	8,0%
SD between-samples Ss	46,0	7,1%

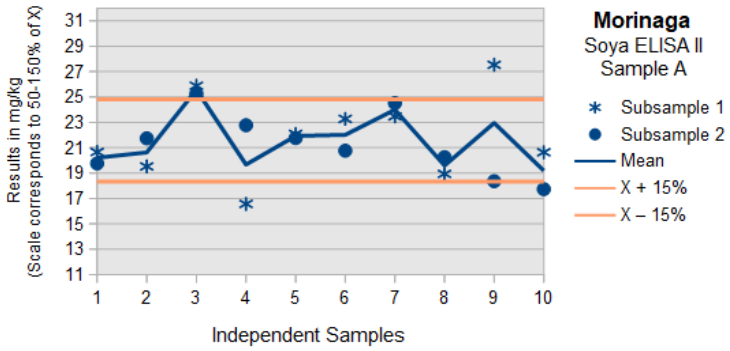


Morinaga Soya ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soy protein 21,6 ± 1,62 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	20,7	19,8	20,2
2	19,5	21,7	20,6
3	25,9	25,3	25,6
4	16,6	22,8	19,7
5	22,1	21,8	21,9
6	23,3	20,8	22,0
7	23,5	24,5	24,0
8	18,9	20,3	19,6
9	27,5	18,4	23,0
10	20,6	17,7	19,2

General average X	21,6	
SD of sample means Sx	2,11	9,8%
SD within-samples Sw	1,91	8,9%
SD between-samples Ss	1,62	7,5%

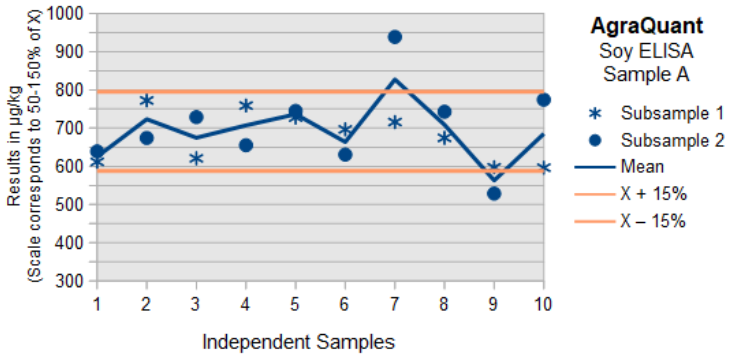


AgraQuant Soy ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soy trypsin inhibitor 691 ± 57,2 mg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	612	639	625
2	772	674	723
3	621	729	675
4	759	655	707
5	728	745	736
6	697	631	664
7	716	938	827
8	674	743	709
9	598	529	563
10	596	774	685

General average X	691	
SD of sample means Sx	69,8	10,1%
SD within-samples Sw	56,5	8,2%
SD between-samples Ss	57,2	8,3%



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 spiking level sample was approx. 0,47 (13°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 7th week of 2021. The testing method was optional. The tests should be finished at 16th April 2021 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters 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 and should be analysed like a normal sample.

Please note the attached information on the proficiency test.
(see documentation, section 5.4 Information on the PT)

2.3 Submission of results

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

All 21 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 level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. No statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].
In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].

All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 18 – 37% 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 sausage (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled sausage (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59
Wheat + Rye	Boiled sausage (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 to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [3].

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of allergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.
The corresponding z-scores were calculated according to 3.5 with the target standard deviation of 25% (see 3.4.3).

4. Results

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

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

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

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 (ELISA-Systems Test Kit Manual: 25,6%).

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%).

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%).

ELISA results reported as **soy trypsin inhibitor (STI)** have been converted to **soy protein** according to the test kit information (Bio-Check Manual: factor 41,7 (600 µg/kg trypsin inhibitor corresponds to 25 mg/kg unheated soy flour)).

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		
4	positive	7,00	negative	<0,2	2/2 (100%)	AQ	
18a	positive	14,5	negative	<0,2	2/2 (100%)	BC	
1	positive	>2,6	negative	<0,26	2/2 (100%)	ES	Result converted °
16	positive	9,28	negative	<2,5	2/2 (100%)	IL	
6	positive	11,0	negative	<0,25	2/2 (100%)	MI-II	
17	positive	13,4	negative	<0,25	2/2 (100%)	MI-II	Result converted °
5	positive	20,3	negative	< 2,5	2/2 (100%)	RS-F	
11	positive		negative		2/2 (100%)	RS-F	
12	positive	17,0	negative	<0,3	2/2 (100%)	RS-F	
14	positive	4,10	negative	<2,5	2/2 (100%)	RS-F	
15	positive	15,7	negative	<0,12	2/2 (100%)	RS-F	
18b	positive	38,3	negative	<2,5	2/2 (100%)	RS-F	
13	positive	15,0	negative	< 0,2	2/2 (100%)	SP	
20	positive		negative		2/2 (100%)	SP	
21	positive		negative		2/2 (100%)	SP	
22	positive		negative		2/2 (100%)	SP	
2	positive	48,0	negative	< 2,5	2/2 (100%)	VT	Result indicated as NFDM?

° calculation see p. 19

	Sample A		Sample B	
Number positive	17		0	
Number negative	0		17	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Casein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4	7,00	-1,5	AQ	
18a	14,5	0,45	BC	
1	>2,6		ES	Result converted °
16	9,28	-0,91	IL	
6	11,0	-0,46	MI-II	
17	13,4	0,16	MI-II	Result converted °
5	20,3	2,0	RS-F	
11			RS-F	
12	17,0	1,1	RS-F	
14	4,10	-2,3	RS-F	
15	15,7	0,76	RS-F	
18b	38,3	6,6	RS-F	Outlier XAll
13	15,0	0,58	SP	
20			SP	
21			SP	
22			SP	
2	48,0	9,2	VT	Outlier XAll, result indicated as NFDM?

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

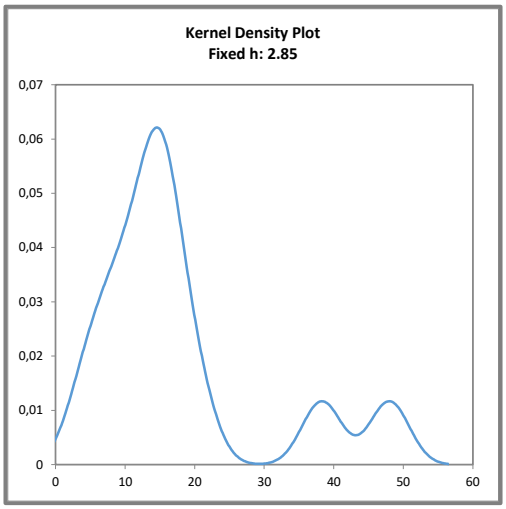


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with two minor side-peaks due to two outlier results.

Characteristics: Quantitative evaluation ELISA Casein

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results°	10
Number of outliers	2
Mean	12,7
Median	14,0
Robust Mean (X_{pt})	**12,8**
Robust standard deviation (S*)	**5,43**
Target range:	
Target standard deviation $\sigma_{pt'}$	**3,85**
lower limit of target range	**5,08**
upper limit of target range	**20,5**
Quotient S/$\sigma_{pt'}$*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*2,15*
Results in the target range	*9*
Percent in the target range	*90*

° without results no. 2 and 18b (excluded)

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 a slightly increased variability of results, with a quotient S/σpt just below 2,0. Due to the large number of different test methods with a small number of individual results each, the evaluation was carried out using the z'-score, taking into account the standard uncertainty.*
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 108% of the spiking level of casein to sample A and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.28).

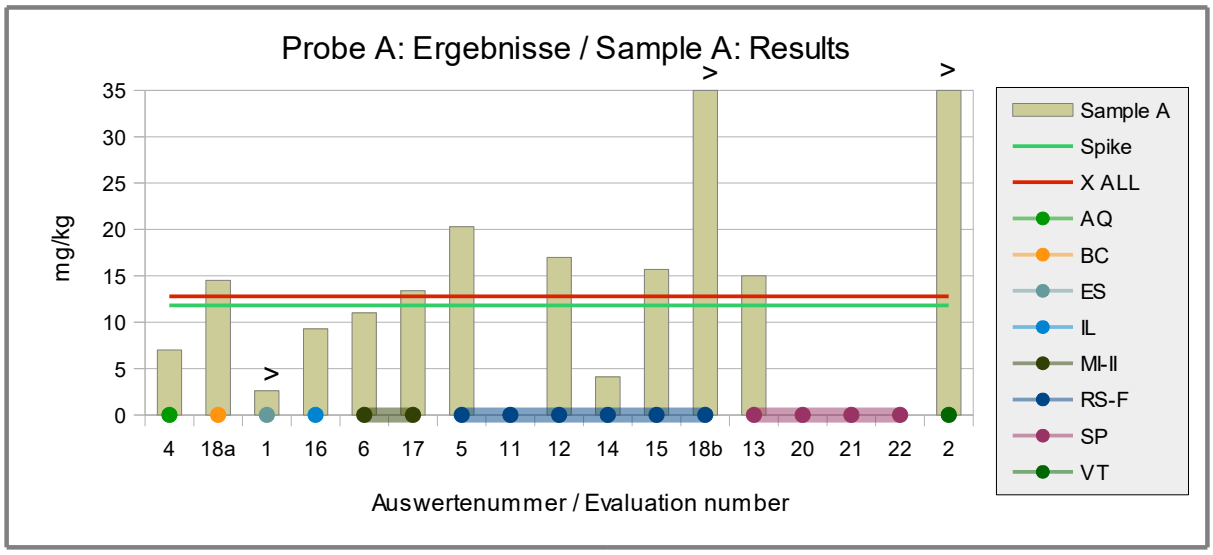


Abb./Fig. 2: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

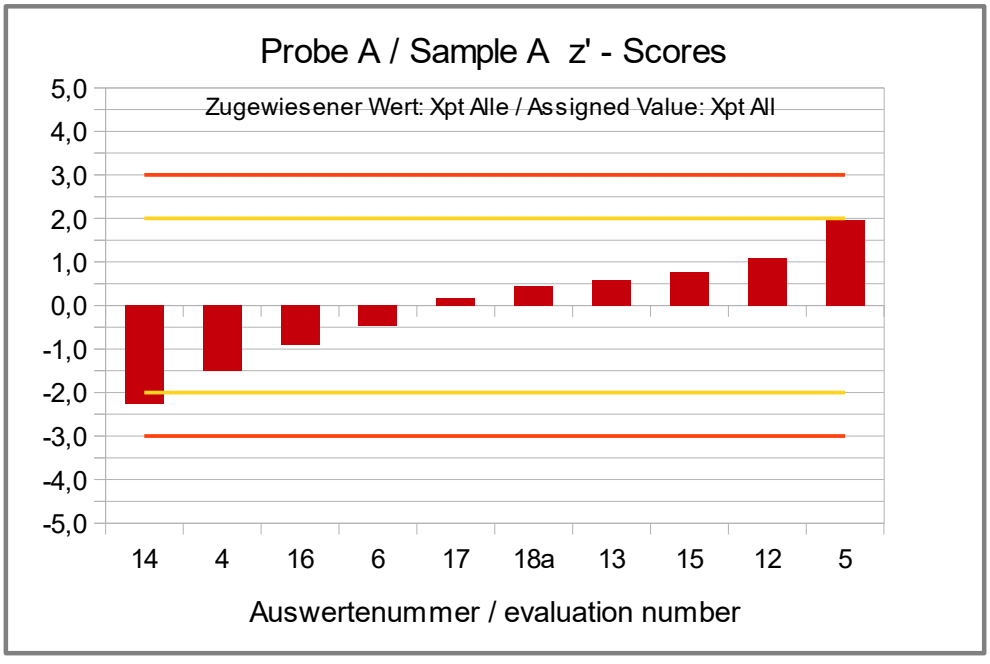


Abb./Fig. 3:
z'-Scores (ELISA Results casein)
Assigned value robust mean of all results

Quantitative valuation of results: Spiking level sample

Evaluation number	Casein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4	12,1	-1,4	AQ	
18a	22,1	0,84	BC	
1	>2,6		ES	Result converted °
16	15,5	-0,62	IL	
6	15,0	-0,72	MI-II	
17	14,2	-0,89	MI-II	Result converted °
5	27,5	2,0	RS-F	
11			RS-F	
12	21,0	0,60	RS-F	
14	14,1	-0,91	RS-F	
15	18,2	-0,02	RS-F	
18b	53,5	7,7	RS-F	Outlier XAll
13	24,0	1,3	SP	
20			SP	
21			SP	
22			SP	
2	53,0	7,6	VT	Outlier XAll, result indicated as NFDM?

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

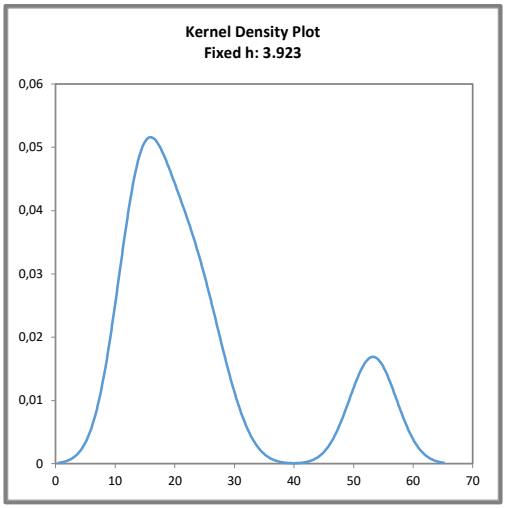


Abb. / Fig. 4:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a minor side-peak due to two outlier results.

Characteristics: Quantitative evaluation ELISA Casein

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results°	10
Number of outliers	2
Mean	18,4
Median	16,8
Robust Mean (X_{pt})	**18,3**
Robust standard deviation (S*)	**5,55**
Target range:	
Target standard deviation σ_{pt}	**4,57**
lower limit of target range	**9,14**
upper limit of target range	**27,4**
Quotient S/σ_{pt}*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*2,19*
Results in the target range	*10*
Percent in the target range	*100*

° *without results no. 2 and 18b (excluded)*

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 a normal variability of results, with a quotient S/σpt 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 125% of the spiking level of casein to the spiking level sample and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.28).

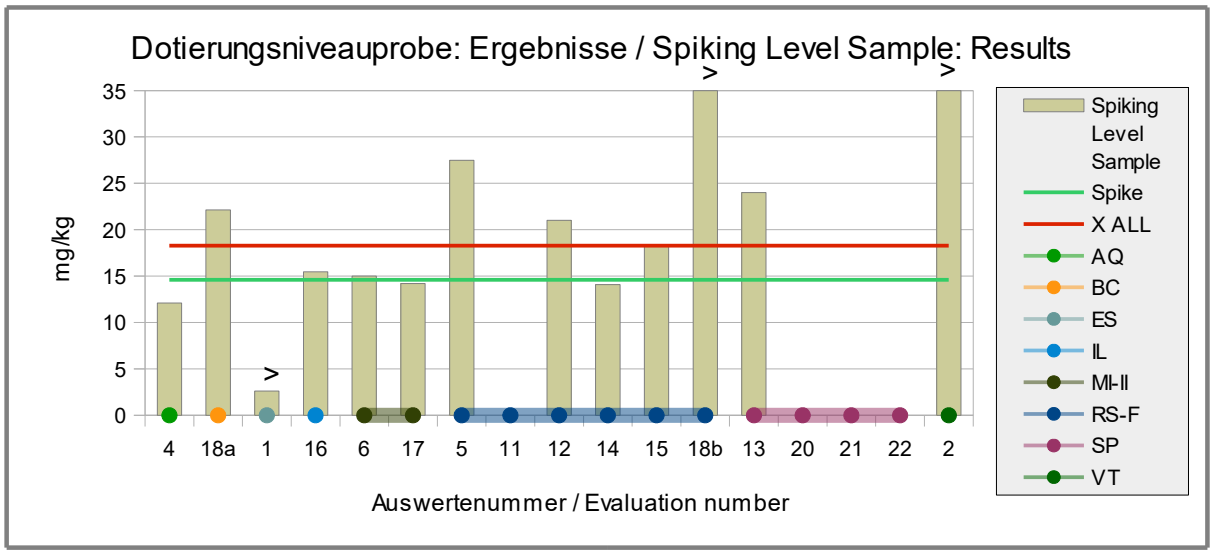


Abb./Fig. 5: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

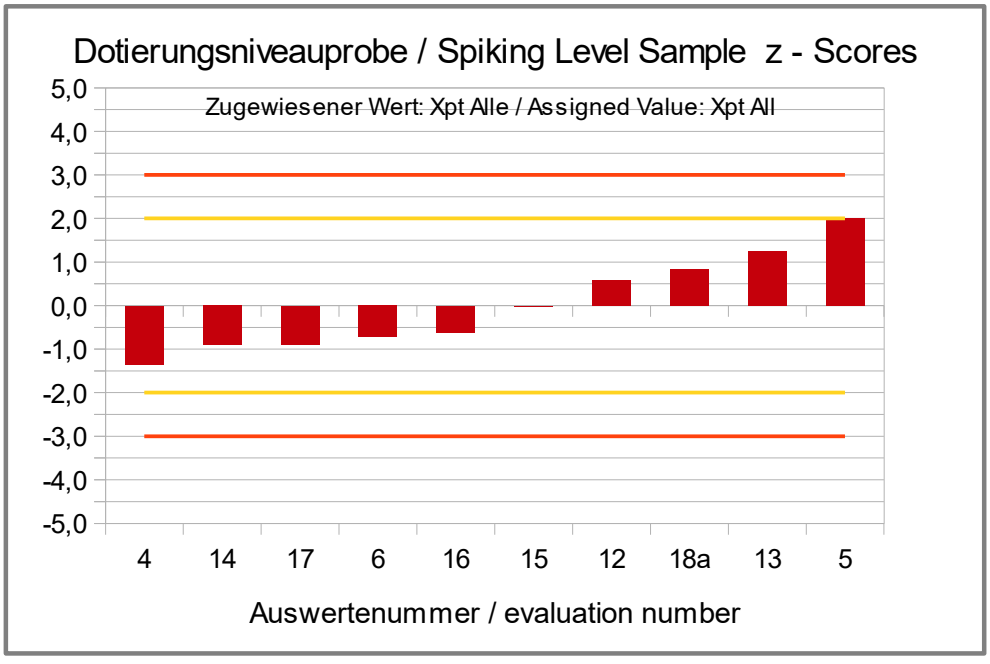


Abb./Fig. 6:
z-Scores (ELISA Results casein)
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA for Casein: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
4	12,1	**83**	-0,68	7,00	**59**	-1,6	AQ	
18a	22,1	152	2,1	14,5	**123**	0,92	BC	
1	>2,6			>2,6			ES	Result converted °
16	15,5	**106**	0,24	9,28	**79**	-0,85	IL	
6	15,0	**103**	0,11	11,0	**93**	-0,27	MI-II	
17	14,2	**97**	-0,11	13,4	**114**	0,54	MI-II	Result converted °
5	27,5	188	3,5	20,3	172	2,9	RS-F	
11							RS-F	
12	21,0	**144**	1,8	17,0	**144**	1,8	RS-F	
14	14,1	**97**	-0,14	4,10	35	-2,6	RS-F	
15	18,2	**125**	1,0	15,7	**133**	1,3	RS-F	
18b	53,5	367	11	38,3	324	9,0	RS-F	
13	24,0	164	2,6	15,0	**127**	1,1	SP	
20							SP	
21							SP	
22							SP	
2	53,0	363	10,52	48,0	407	12	VT	Result indicated as NFDM?

° Calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	7	Number in RA	8
Percent in RA	58	Percent in RA	67

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

58% (7) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 67% (8) of the recovery rates were within the range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

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		
18	positive	6,88	negative	<0.4	2/2 (100%)	AQ	
14	positive	6,20	negative	<2,5	2/2 (100%)	RS-F	
6	-		-			SP	
21	positive		negative		2/2 (100%)	SP	
3	positive	16,2	negative	n.n.	2/2 (100%)	VT	
17	positive	8,81	negative	<0,88	2/2 (100%)	VT	Result converted °
19	positive	13,7	negative	<0,88	2/2 (100%)	VT	Result converted °

° calculation see p. 19

	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

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

Quantitative valuation of ELISA-results: Sample A

Due to the limited number of results, the following evaluation is only given for information:

Evaluation number	Milk Protein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
18	6,88	-0,92	AQ	
14	6,20	-1,1	RS-F	
6			SP	
21			SP	
3	16,2	1,5	VT	
17	8,81	-0,41	VT	Result converted °
19	13,7	0,88	VT	Result converted °

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Note:
Due to the low number of < 8 results a kernel density estimation could not be carried out.

Characteristics: Quantitative evaluation ELISA Milk Protein

Due to the limited number of results, the following evaluation is only given for information:

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	10,4
Median	8,81
Robust Mean (X_{pt})	**10,4**
Robust standard deviation (S*)	**4,98**
Target range:	
Target standard deviation $\sigma_{pt'}$	**3,80**
lower limit of target range	**2,76**
upper limit of target range	**18,0**
Quotient S/$\sigma_{pt'}$*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*2,78*
Results in the target range	*5*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a slightly increased variability of results, with a quotient S/σpt just below 2,0. Due to different test methods with a small number of individual results each, the evaluation was carried out using the z'-score, taking into account the standard uncertainty.*
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 70% of the spiking level of milk protein to sample A and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.36).

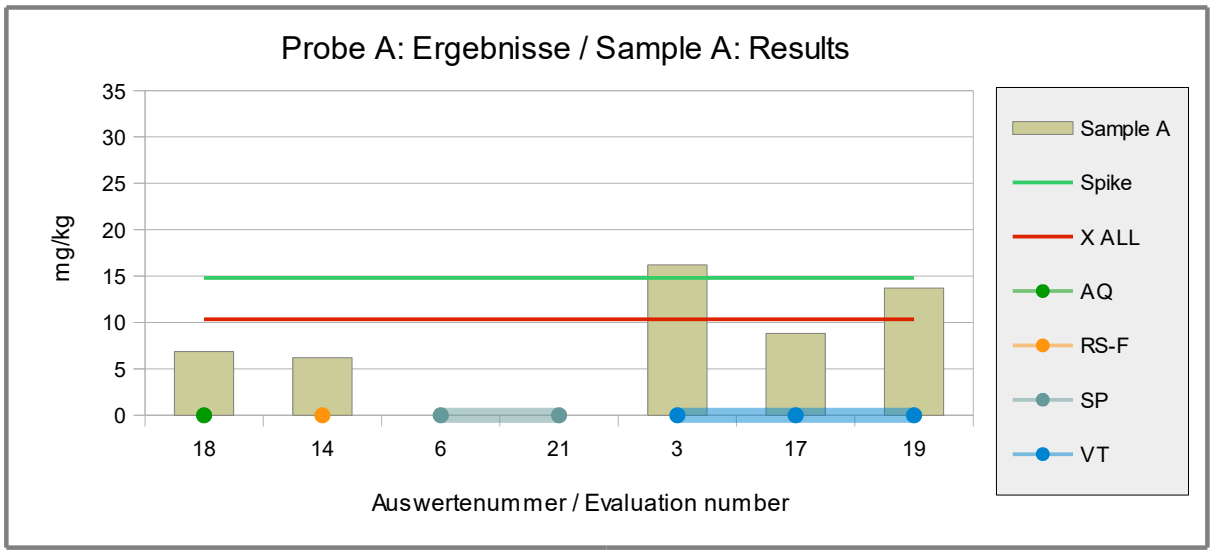


Abb./Fig. 7: ELISA Results milk protein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

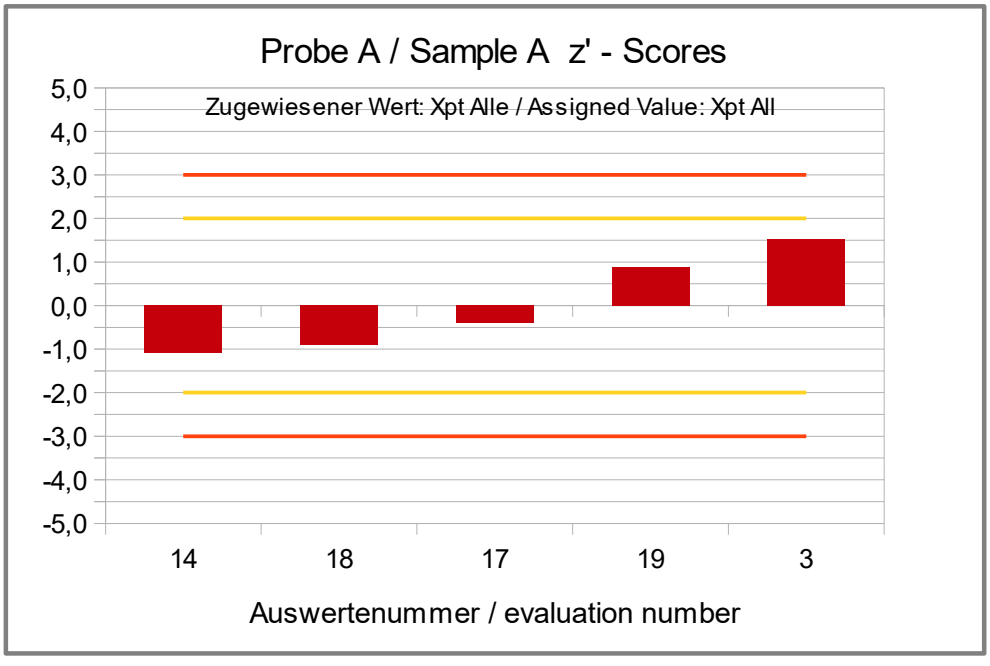


Abb./Fig. 8:
z'-Scores (ELISA Results milk protein)
Assigned value robust mean of all results

Quantitative valuation ELISA: Spiking level sample

Due to the limited number of results, the following evaluation is only given for information:

Evaluation number	Milk Protein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
18	7,48	-1,1	AQ	
14	4,70	-1,6	RS-F	
6			SP	
21			SP	
3	20,1	1,3	VT	
17	15,8	0,50	VT	Result converted °
19	17,6	0,83	VT	Result converted °

° calculation see p. 19

Methoden:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Note:
Due to the low number of < 8 results a kernel density estimation could not be carried out.

Characteristics: Quantitative evaluation ELISA Milk Protein

Due to the limited number of results, the following evaluation is only given for information:

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	5
Number of outliers	0
Mean	13,1
Median	15,8
Robust Mean (X_{pt})	**13,1**
Robust standard deviation (S*)	**7,58**
Target range:	
Target standard deviation $\sigma_{pt'}$	**5,36**
lower limit of target range	**2,41**
upper limit of target range	**23,9**
Quotient S/$\sigma_{pt'}$*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*4,24*
Results in the target range	*5*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The evaluation of all methods showed a slightly increased variability of results, with a quotient S/σpt just below 2,0. Due to different test methods with a small number of individual results each, the evaluation was carried out using the z'-score, taking into account the standard uncertainty.*
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 72% of the spiking level of milk protein to the spiking level sample and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of casein" p.36).

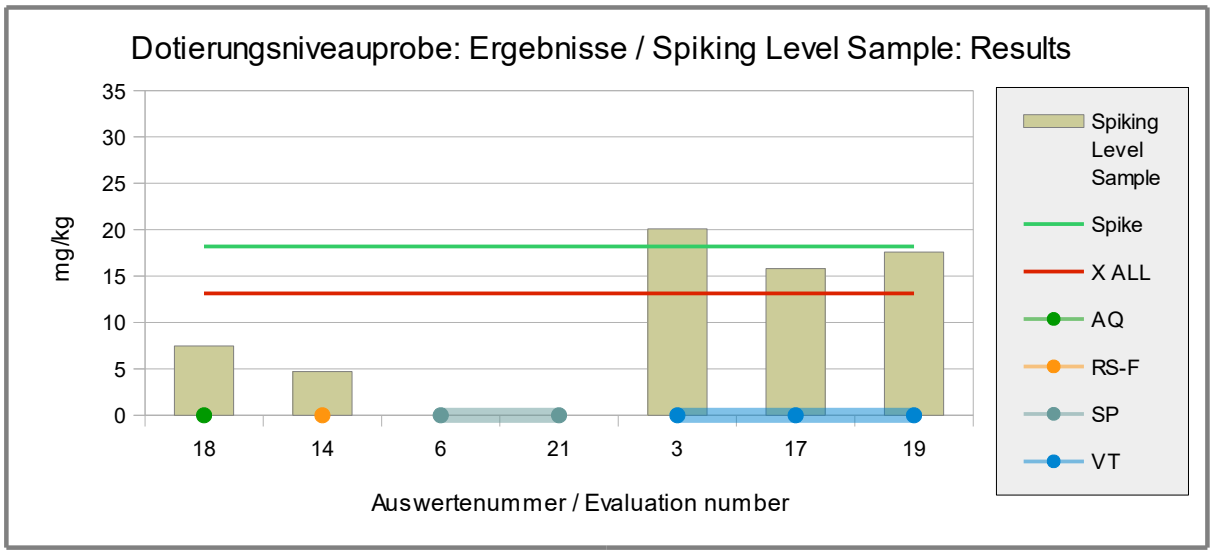


Abb./Fig. 9: ELISA Results milk (as milk protein)
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

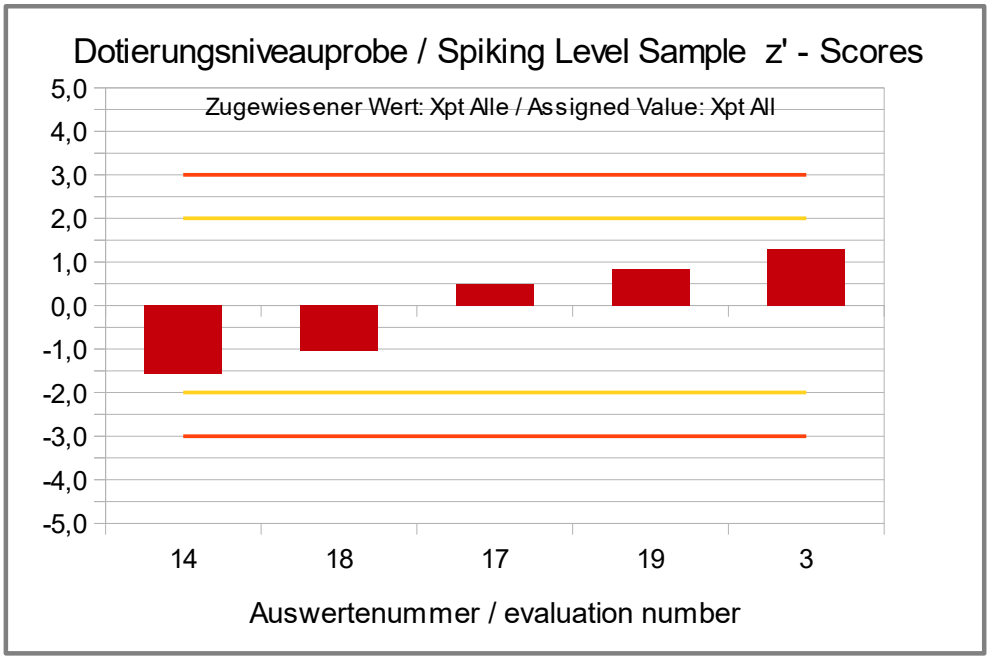


Abb./Fig. 10:
z-Scores (ELISA results milk as milk protein)
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA *Milk Protein:* Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
18	7,48	41	-2,4	6,88	46	-2,1	AQ	
14	4,70	26	-3,0	6,20	42	-2,3	RS-F	
6							SP	
21							SP	
3	20,1	**110**	0,42	16,2	**109**	0,38	VT	
17	15,8	**87**	-0,53	8,81	**60**	-1,6	VT	Result converted °
19	17,6	**97**	-0,13	13,7	**93**	-0,30	VT	Result converted °

° calculation see p. 19

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

Methods:
AQ = AgraQuant, RomerLabs
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:

3 participants obtained for the spiking level sample as well as for the spiked food matrix sample A recovery rates by ELISA methods within the range of the AOAC-recommendation of 50-150%.
The related z-scores are based on the target standard deviation of 25%.

4.1.3 ELISA Results: other

One participant submitted results for β-lactoglobulin. They are given in the documentation part.

4.1.4 PCR Results: Milk

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		
19	positive		negative		2/2 (100%)	div	DNA Cow

	Sample A		Sample B	
Spiking	positive		negative	

Methods:
div = not indicated / other method

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

Qualitative valuation PCR: Spiking Level Sample

Evaluation number	Milk	Milk	Method	Remarks
	pos/neg	[mg/kg]		
19	positive		div	DNA Cow

Spiking Level Sample	
Spiking	positive

Methods:
div = not indicated / other method

Comment:
The results is in qualitative agreement with the spiking level sample.

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		
4	positive	5,45	negative	<0,78	2/2 (100%)	BC	Result converted °
6	positive	21,0	negative	<1,25	2/2 (100%)	MI-II	
12	positive	27,0	negative	<0,3	2/2 (100%)	MI-II	
1	positive	>20	negative	<2,5	2/2 (100%)	RS-F	
5	positive	> 20	negative	< 2,5	2/2 (100%)	RS-F	
9	positive	18,1	negative	<2,5	2/2 (100%)	RS-F	
10	positive	17,0	negative	<2,5	2/2 (100%)	RS-F	
11	positive		negative		2/2 (100%)	RS-F	
14	positive	10,1	negative	<2,5	2/2 (100%)	RS-F	
16	positive	35,7	negative	<2,5	2/2 (100%)	RS-F	
18a	positive	31,0	negative	<2.5	2/2 (100%)	RS-F	
19a	positive	27,0	negative	<2.5	2/2 (100%)	RS-F	
13	positive	11,0	negative	<2	2/2 (100%)	SP	
21	positive		negative		2/2 (100%)	SP	
2	positive	11,8	negative	< 1,2	2/2 (100%)	VT	Result converted °
3	positive	16,6	negative	n.n.	2/2 (100%)	VT	
7	positive	31,0	negative	0	2/2 (100%)	VT	Result indicated as soy flour!?
8	positive	16,0	negative	0,16	2/2 (100%)	VT	Result converted °
17	positive	12,0	negative	< 1,2	2/2 (100%)	VT	Result converted °
18b	positive	12,1	negative	< 1,2	2/2 (100%)	VT	Result converted °
19b	positive	13,6	negative	< 1,2	2/2 (100%)	VT	Result converted °

° calculation see p. 19

	Sample A		Sample B	
Number positive	21		0	
Number negative	0		21	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

BC = BioCheck ELISA
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Soy Protein	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
4	5,45	-2,8			BC	Result converted °
6	21,0	0,56			MI-II	
12	27,0	1,9			MI-II	
1	>20				RS-F	
5	> 20				RS-F	
9	18,1	-0,07	-0,87		RS-F	
10	17,0	-0,31	-1,1		RS-F	
11					RS-F	
14	10,1	-1,81	-2,3		RS-F	
16	35,7	3,75	2,2		RS-F	
18a	31,0	2,7	1,4		RS-F	
19a	27,0	1,86	0,66		RS-F	
13	11,0	-1,61			SP	
21					SP	
2	11,8	-1,44		-0,75	VT	Result converted °
3	16,6	-0,4		0,58	VT	
7	31,0	2,7		4,5	VT	Result indicated as soy flourl?
8	16,0	-0,53		0,41	VT	Result converted °
17	12,0	-1,40		-0,69	VT	Result converted °
18b	12,1	-1,4		-0,66	VT	Result converted °
19b	13,6	-1,0		-0,25	VT	Result converted °

° calculation see p. 19

Methods:

BC = BioCheck ELISA
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

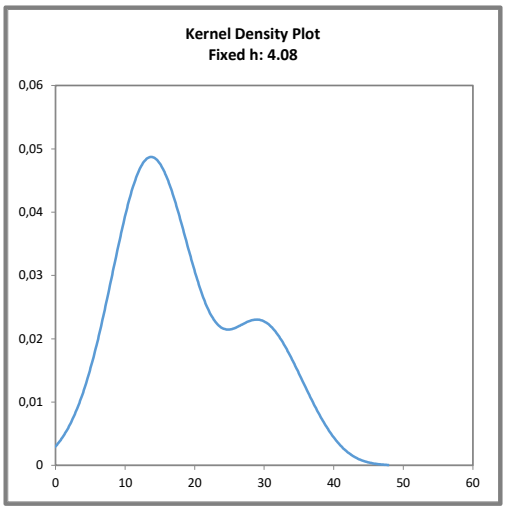


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 major peak at approx. 15 mg/kg and a side-peak at approx. 30 mg/kg due to single results of different methods.

Characteristics: Quantitative evaluation ELISA Soy Protein

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*	**Method VT** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ iRS\text{-}F}}$	$X_{pt_{METHOD\ iRS\text{-}F}}$
Number of results	17	6	7
Number of outliers	0	0	0
Mean	18,6	23,2	16,2
Median	16,6	22,5	13,6
Robust Mean (X_{pt})	**18,4**	**23,2**	**14,5**
Robust standard deviation (S*)	**9,52**	**11,0**	**3,33**
Target range:			
Target standard deviation $\sigma_{pt'}$ or σ_{pt}	**5,439**	**5,79**	**3,63**
lower limit of target range	**7,56**	**11,60**	**7,26**
upper limit of target range	**29,3**	**34,7**	**21,8**
Quotient S/$\sigma_{pt'}$ or S*/σ_{pt}*	*1,8*	*1,9*	*0,92*
Standard uncertainty $U_{(Xpt)}$	*2,89*	*5,60*	*1,57*
Results in the target range	*13*	*4*	*6*
Percent in the target range	*76*	*67*	*86*

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 ELISA methods the evaluation of all results (X_{ALL}) was done by z'-score considering the standard uncertainty. The quotient S/$\sigma_{pt'}$ was then 1,8. The quotients S*/σ_{pt} of the both other evaluations (RS-F and VT) were below 2,0. The robust standard deviations are partly 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.*

The robust means of the evaluations were 81% (X_{ALL}), 103% ($X_{RS\text{-}F}$) and 64% (X_{VT}) of the spiking level of soy protein to sample A and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soyprotein" p.48).

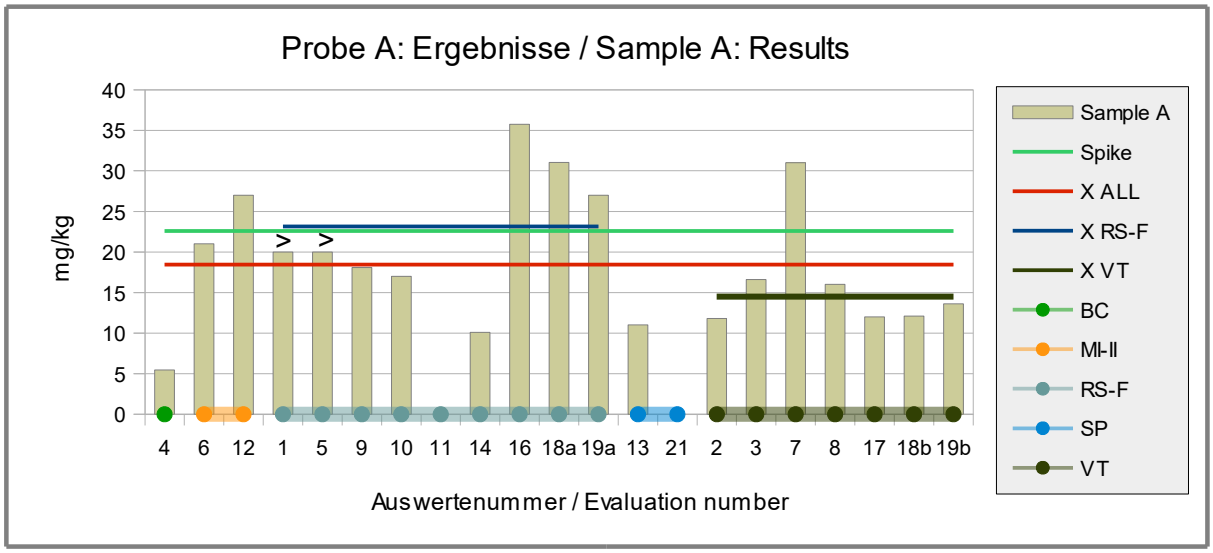


Abb./Fig. 12: ELISA Results soya (as soy protein)
green line = Spiking level
red line = Assigned value robust mean of all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

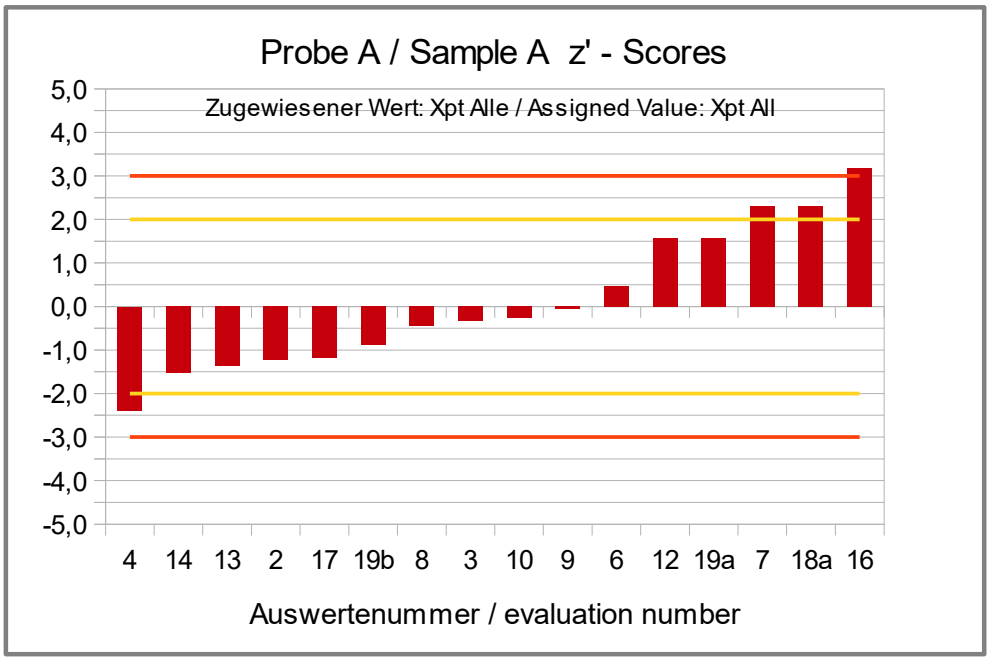


Abb./Fig. 13:
z'-Scores (ELISA Results soy protein)
Assigned value robust mean of all results

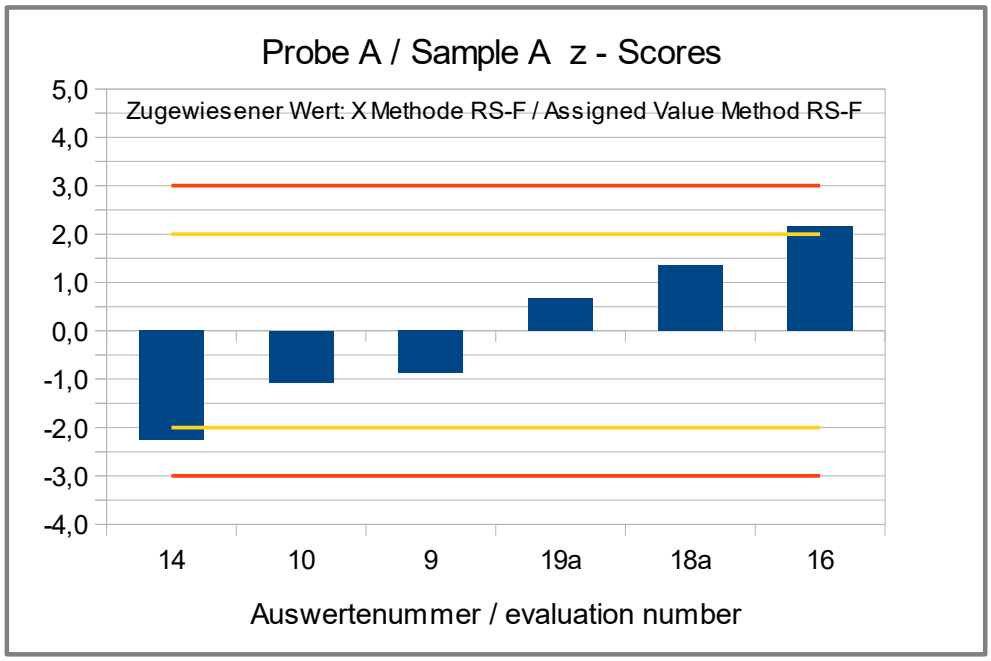


Abb./Fig. 14:
z-Scores ELISA Results as soy protein, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

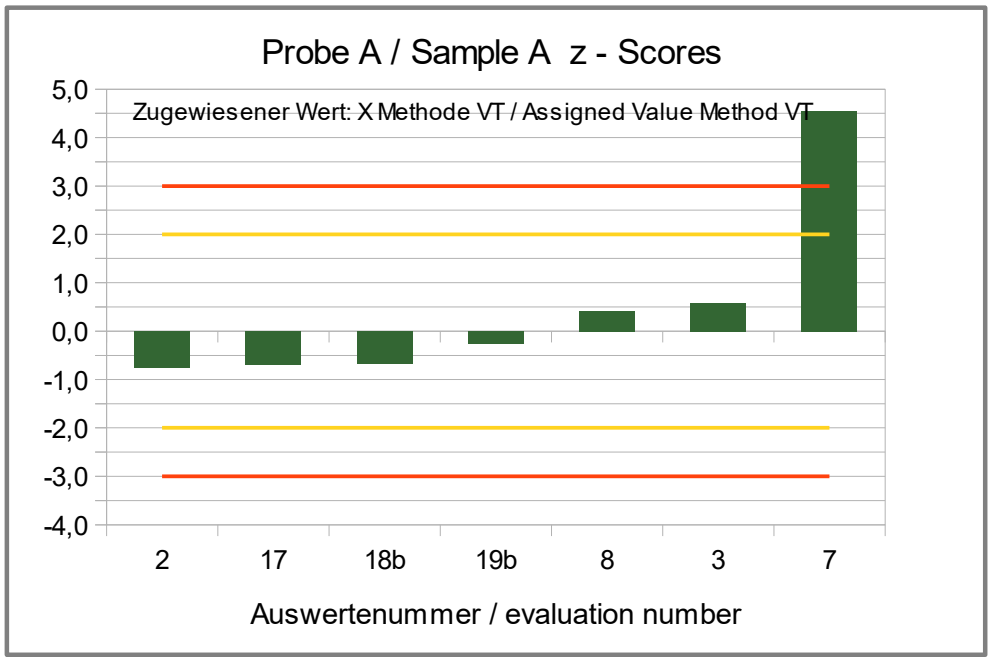


Abb./Fig. 15:
z-Scores ELISA Results as soy protein, Assigned value robust mean of results method VT (Veratox, Neogen)

Quantitative Valuation of results: Spiking level sample

Evaluation number	Soy Protein	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	z-Score Xpt_{VT}	Method	Remarks
	[mg/kg]					
4	8,11	-2,0			BC	Result converted °
6	17,0	-0,70			MI-II	
12	33,0	1,6			MI-II	
1	>20				RS-F	
5	>20				RS-F	
9	11,8	-1,4	-2,4		RS-F	
10					RS-F	
11					RS-F	
14	12,7	-1,3	-2,3		RS-F	
16	50,4	4,1	2,9		RS-F	
18a	30,3	1,2	0,15		RS-F	
19a	41,0	2,7	1,6		RS-F	
13	26,0	0,59			SP	
21					SP	
2	12,2	-1,4		-0,82	VT	Result converted °
3	17,9	-0,57		0,66	VT	
7	44,0	3,2		7,5	VT	Result indicated as soy flourl?
8	17,4	-0,64		0,53	VT	Result converted °
17	9,20	-1,8		-1,6	VT	Result converted °
18b	14,6	-1,0		-0,20	VT	Result converted °
19b	13,2	-1,2		-0,56	VT	Result converted °

° calculation see p. 19

Methods:

BC = BioCheck ELISA
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

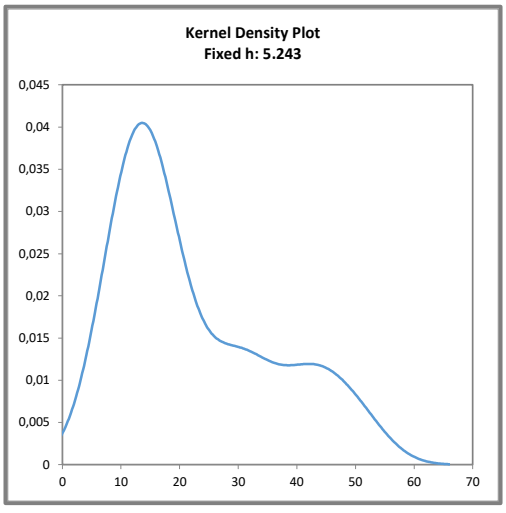


Abb. / Fig. 16:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 major peak at approx. 15 mg/kg and a few single results of different methods in the range of >25 mg/kg.

Characteristics: Quantitative evaluation ELISA Soya (as soy protein)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*	**Method VT** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ iRS\text{-}F}}$	$X_{pt_{METHOD\ iRS\text{-}F}}$
Number of results	16	5	7
Number of outliers	0	0	-
Mean	22,4	29,3	18,4
Median	17,2	30,3	14,6
Robust Mean (X_{pt})	**21,9**	**29,3**	**15,4**
Robust standard deviation (S*)	**13,9**	**19,3**	**5,13**
Target range:			
Target standard deviation $\sigma_{pt'}$ or σ_{pt}	**6,99**	**7,31**	**3,84**
lower limit of target range	**7,89**	**14,6**	**7,68**
upper limit of target range	**35,8**	**43,9**	**23,0**
Quotient S/$\sigma_{pt'}$ or S*/σ_{pt}*	*2,0*	*2,6*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*4,35*	*10,8*	*2,42*
Results in the target range	*13*	*2*	*6*
Percent in the target range	*81*	*40*	*86*

Comments to the statistical characteristics and assigned values:

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

The distributions of results of all methods as well as of method RS-F showed an increased variability with quotients S/σ_{pt} of above 2,0. Therefore the evaluation of results of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then 2,0.*
For the evaluation of the results of method RS-F, this was not done because the target range would otherwise be unsuitably large. The separate evaluation of the RS-F results was therefore done for information only. The distribution of results of method VT showed a normal variability with a quotient S/σ_{pt} < 2,0.*
The robust standard deviations are partly 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.

The robust means of the evaluations were 78% (X_{ALL}), 98% ($X_{RS\text{-}F}$) and 61% (X_{VT}) of the spiking level of soy protein to the spiking level sample and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soyprotein" p.48).

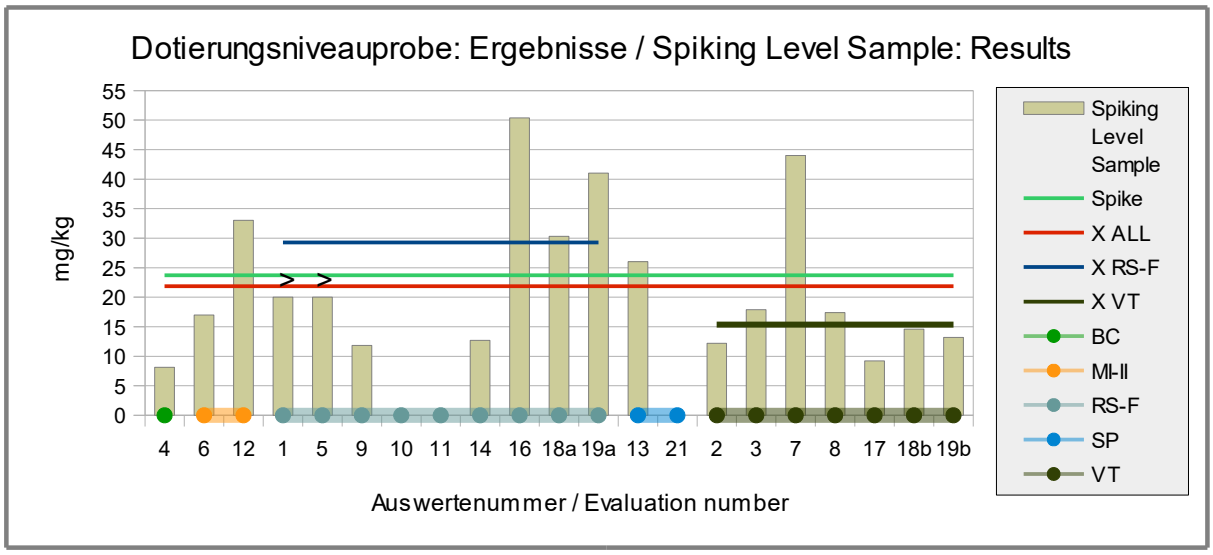


Abb./Fig. 17: ELISA Results soya (as soy protein)
green line = Spiking level
red line = Assigned value robust mean of all results
blue line = Assigned value robust mean method RS-F
dark green = Assigned value robust mean method VT
round symbols = Applied methods (see legend)

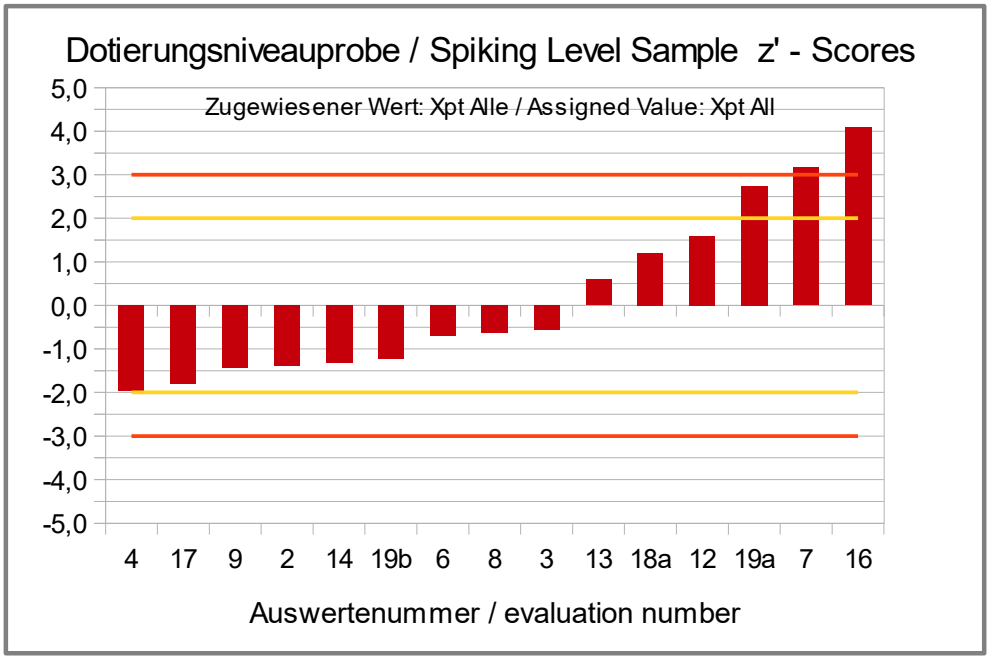


Abb./Fig. 18:
z'-Scores (ELISA Results soy protein)
Assigned value robust mean of all results

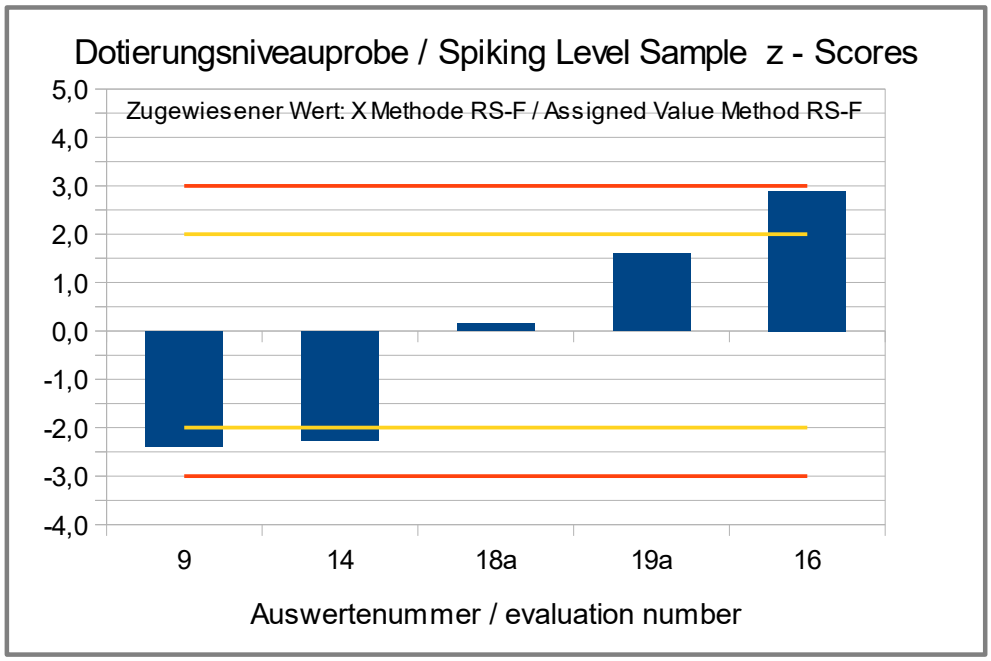


Abb./Fig. 19:
z-Scores ELISA Results as soy protein, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

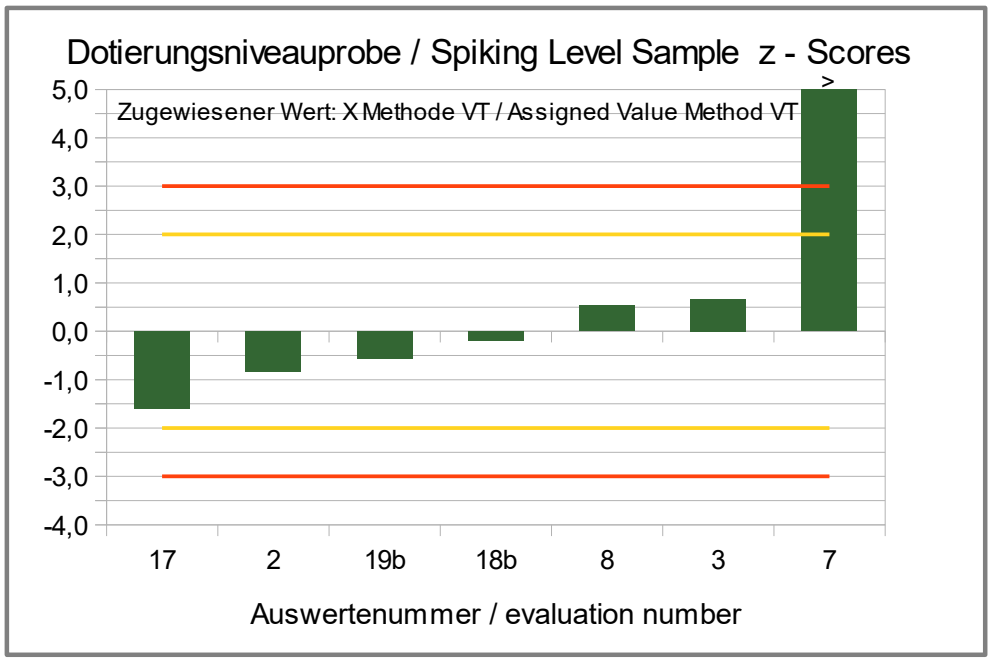


Abb./Fig. 20:
z-Scores ELISA Results as soy protein, Assigned value robust mean of results method VT (Veratox, Neogen)

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]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
4	8,11	34	-2,6	5,45	24	-3,0	BC	Result converted °
6	17,0	**72**	-1,1	21,0	**93**	-0,28	MI-II	
12	33,0	**139**	1,57	27,0	**119**	0,78	MI-II	
1	>20			>20			RS-F	
5	>20			>20			RS-F	
9	11,8	**50**	-2,0	18,1	**80**	-0,80	RS-F	
10				17,0	**75**	-0,99	RS-F	
11							RS-F	
14	12,7	**54**	-1,9	10,1	45	-2,2	RS-F	
16	50,4	213	4,5	35,7	158	2,3	RS-F	
18a	30,3	**128**	1,1	31,0	**137**	1,5	RS-F	
19a	41,0	173	2,9	27,0	**119**	0,78	RS-F	
13	26,0	**110**	0,39	11,0	49	-2,1	SP	
21							SP	
2	12,2	**51**	-1,9	11,8	**52**	-1,9	VT	Result converted °
3	17,9	**76**	-1,0	16,6	**73**	-1,1	VT	
7	44,0	186	3,4	31,0	**137**	1,5	VT	Result indicated as soy flour!?
8	17,4	**73**	-1,1	16,0	**71**	-1,2	VT	Result converted °
17	9,20	39	-2,4	12,0	**53**	-1,9	VT	Result converted °
18b	14,6	**62**	-1,5	12,1	**54**	-1,9	VT	Result converted °
19b	13,2	**56**	-1,8	13,6	**60**	-1,6	VT	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**13**
Percent in RA	**69**	Percent in RA	**76**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BC = BioCheck ELISA

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

69% (11) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 76% (13) of the recovery rates were within the range of acceptance.

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

4.2.2 PCR Results: 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		
20	negativ		negativ		1/2 (50%)	GR	no positive sample detected
22	negativ		negativ		1/2 (50%)	GR	no positive sample detected
5	positiv		negativ		2/2 (100%)	SFA	
21	positiv		negativ		2/2 (100%)	SFA	
18	positiv	20,9	negativ	<1	2/2 (100%)	SFA-ID	
11	positiv		negativ		2/2 (100%)	SFA-Q	
6	positiv		negativ		2/2 (100%)	div	
19	positiv		negativ		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	6		0	
Number negative	2		8	
Percent positive	75		0	
Percent negative	25		100	
Consensus value	positiv		negativ	

Methods:
GR = SPECIALfinder Assay, real time PCR, Generon
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. Two participants obtained negative results for sample A (method GR).

Quantitative Valuation PCR: Sample A

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

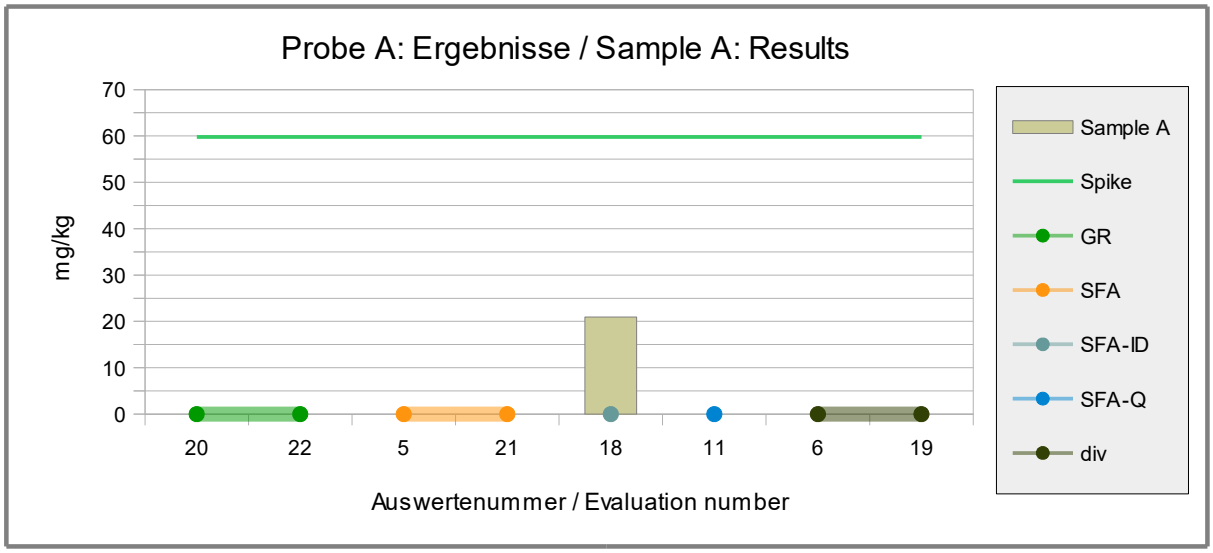


Abb./Fig. 21: PCR Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

Qualitative valuation PCR: Spiking Level Sample

An evaluation of the quantitative results was not carried out because too few results were available

Evaluation number	Soya	Soya	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
20	positive			GR	
22	positive			GR	
5	positive			SFA	
21	positive			SFA	
18	positive	34,6		SFA-ID	
11	positive			SFA-Q	
6	-			div	
19	positive			div	

	Sample A	
Number positive	7	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:

GR = SPECIALfinder Assay, real time PCR, Generon
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

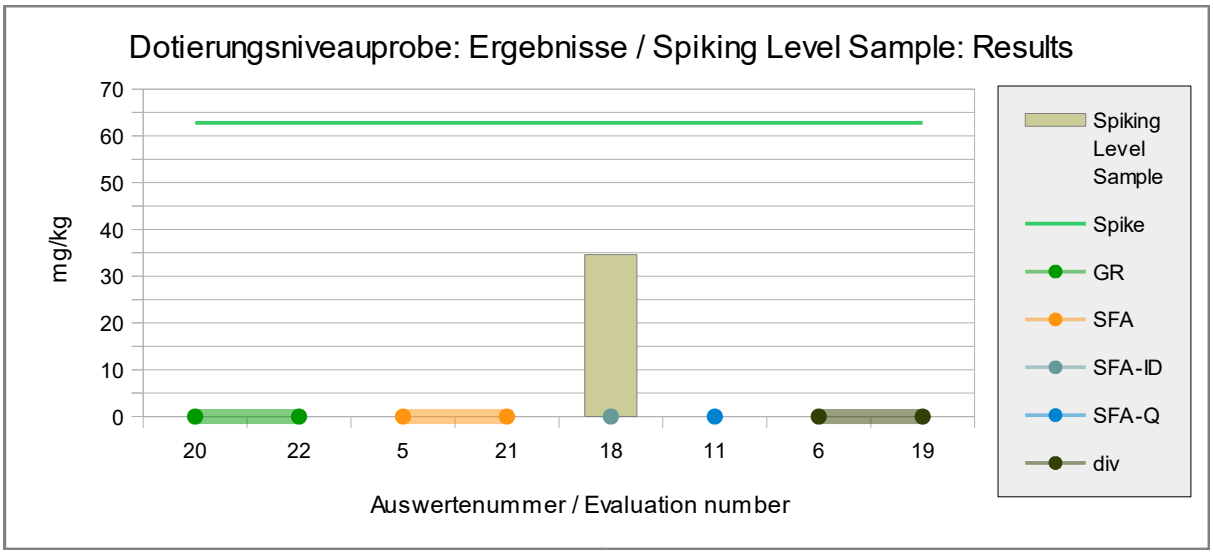


Abb./Fig. 22: PCR Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for Soya: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
20							GR	
22							GR	
5							SFA	
21							SFA	
18	34,6	**55**	-1,8	20,9	35	-2,6	SFA-ID	
11							SFA-Q	
6							div	
19							div	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	0
Percent in RA	100	Percent in RA	0

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

GR = SPECIALfinder Assay, real time PCR, Generon

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

One participant submitted quantitative PCR results for soya. The recovery rate for the spiking level sample was in the range of the AOAC requirement of 50-150%.

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

4.3 Participant z-Scores: overview table

Z-Scores for the assigned values from participants results (consensus values)

Evaluation number	ELISA Casein **Xpt** (div. Methods)		ELISA Milk Protein **Xpt** (div. Methods)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1				
2	9,2	7,6		
3			1,5	1,3
4	-1,50	-1,35		
5	2,00	2,02		
6	-0,46	-0,72		
11				
12	1,10	0,60		
13	0,58	1,25		
14	-2,3	-0,91	-1,1	-1,6
15	0,76	-0,02		
16	-0,91	-0,62		
17	0,16	-0,89	-0,41	0,50
18			-0,92	-1,1
18a	0,45	0,84		
18b	6,6	7,7		
19			0,88	0,83
20				
21				
22				

Evaluation number	ELISA Soyprotein **Xpt** (div. Methods)		ELISA Soyprotein **Xpt** (Method: RS-F)		ELISA Soyprotein **Xpt** (Method: VT)	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1						
2	-1,44	-1,38			-0,75	-0,82
3	-0,40	-0,57			0,58	0,66
4	-2,8	-1,97				
5						
6	0,56	-0,70				
7	2,7	3,2			4,5	7,5
8	-0,53	-0,64			0,41	0,53
9	-0,07	-1,44	-0,87	-2,4		
10	-0,31		-1,06			
11						
12	1,86	1,59				
13	-1,61	0,59				
14	-1,81	-1,31	-2,3	-2,3		
16	3,8	4,1	2,2	2,9		
17	-1,40	-1,81			-0,69	-1,60
18a	2,7	1,21	1,36	0,15		
18b	-1,38	-1,04			-0,66	-0,20
19a	1,86	2,7	0,66	1,61		
19b	-1,05	-1,24			-0,25	-0,56
21						

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

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

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Casein		ELISA Milk Protein	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1				
2	12	11		
3			0,38	0,42
4	-1,6	-0,68		
5	2,9	3,5		
6	-0,27	0,11		
11				
12	1,8	1,8		
13	1,1	2,6		
14	-2,6	-0,14	-2,3	-3,0
15	1,3	1,0		
16	-0,85	0,24		
17	0,54	-0,11	-1,6	-0,53
18			-2,1	-2,4
18a	0,92	2,1		
18b	9,0	11		
19			-0,30	-0,13
20				
21				
22				

Evaluation number	ELISA Soyprotein		PCR Soya	
	Sample A	Spiking Level Sample	Sample A	Spiking Level Sample
1				
2	-1,9	-1,9		
3	-1,1	-1,0		
4	-3,0	-2,6		
5				
6	-0,28	-1,1		
7	1,5	3,4		
8	-1,2	-1,1		
9	-0,80	-2,0		
10	-0,99			
11				
12	0,78	1,6		
13	-2,1	0,39		
14	-2,2	-1,9		
16	2,3	4,5		
17	-1,9	-2,4		
18			-2,6	-1,8
18a	1,5	1,1		
18b	-1,9	-1,5		
19a	0,78	2,9		
19b	-1,6	-1,8		
21				

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: ß-Lactoglobulin

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	%		Test-Kit + Manufacturer
MI-II	6		positive	0,83	negative	<0,031	positive	1,1	0,031	0,031		Beta-Lactoglobulin	Morinaga BLG ELISA Kit II

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
MI-II	6	detects ß-Lactoglobulin	As per kit instructions	yes	x 10 = total milk protein

5.1.2 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	%		Test-Kit + Manufacturer
AQ	4	09.03.21	positive	7	negative	<0.2	positive	12,1	0,2	0,2	50	Casein	AgraQuant Casein COKAL 1200, RomerLabs
BC	18a	26.02.21	positive	14,51	negative	<0.2	positive	22,14	0,2	0,2	30,39	Casein	BioCheck ELISA Milk-Check (Casein)
ES	1	05.03.21	positive	>10	negative	<1	positive	>10		1		Skimmed milk powder	ELISA Systems Casein ESCASPRD-48
IL	16	15.03.21	positive	9,28	negative	<2,5	positive	15,46	0,4	2,5	-	Casein	Immunolab Casein ELISA
MI-II	6		positive	11	negative	<0,25	positive	15	0,25	0,25		Casein	Morinaga Casein ELISA Kit
MI-II	17	31/3	-	16,7	-	<0.31	-	17,7		0,31		food	Selection Casein-Kits: Morinaga
RS-F	5		positive	20,3	negative	< 2,5	positive	27,5		2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	11	25.03.21	positive		negative		positive		0,5	0,5		Please select!	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	12	15.04.21	-	17	-	<0,3	-	21	0,3	0,6		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	14	25.02.21	positive	4,1	negative	No detectado,<2,5	positive	14,1		2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	15	14.04.21	positive	15,7	negative	<0,12	positive	18,2	0,12	0,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	18b	23.02.21	positive	38,28	negative	<2.5	positive	53,53	2,5	2,5	25,48	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
SP	13	23.03.21	positive	15	negative	< 0.2	positive	24	0.04	0.2		Casein	SensiSpec ELISA Casein, Eurofins
SP	20	08.04.21	positive		negative		positive					Please select!	SensiSpec ELISA Casein, Eurofins
SP	21		positive		negative		positive		0,04	0,2	30	Please select!	SensiSpec ELISA Casein, Eurofins
SP	22	08. Apr	positive		negative		positive					Please select!	SensiSpec ELISA Casein, Eurofins
VT	2	15.03.21	positive	48	negative	< 2,5	positive	53		2,5		Casein	Veratox Casein Allergen, Neogen

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

Continuation ELISA Casein:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	4		0.5g sample/10ml kit extraction buffer/15mins at 60C	yes	
BC	18a	As Per Kit Instructions	As Per Kit Instructions	Yes	
ES	1	Caseín - alfa-S1		no	
IL	16			no	
MI-II	6	detects Casein	As Per Kit Instructions	yes	x 1,24 = total milk protein
MI-II	17				
RS-F	5			yes	
RS-F	11			yes	
RS-F	12			yes	
RS-F	14			No	
RS-F	15			no	
RS-F	18b	As Per Kit Instructions	As Per Kit Instructions	Yes	
SP	13				
SP	20			no	
SP	21				
SP	22			no	
VT	2		1g in 125 mL PBS (supplied by the kit) / 15 minutes / 60°C	yes	

5.1.3 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	%		Test-Kit + Manufacturer
AQ	18	26.02.21	positive	6,88	negative	<0.4	positive	7,48	0,4	0,4	30	Milk proteins, total	AgraQuant ELISA Milk COKAL2448, RomerLabs
RS-F	14	25.02.21	positive	6,2	negative	No detectado,<2,5	positive	4,7		2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
SP	6		-		-		-					Milk proteins, total	SensiSpec ELISA Milk, Eurofins
SP	21		positive		negative		positive		0,05	0,4	30	Please select!	SensiSpec ELISA Milk, Eurofins
VT	3	09.03.21	-	16,2	-	n.n.	-	20,1	0,4	0,9	40	Milk proteins, total	Veratox Total Milk Allergen, Neogen
VT	17	16/4	-	25,1	-	<2.5	-	44,9		2,5		food	Selection Milk-Kits: Neogen
VT	19	21.04.2021, 23.04.2021	positive	39	negative	<2.5	positive	50	2,5	2,5		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 no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	18	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	14			yes	0,9 mg/Kg for sample A and No detectado,<0,4 for sample B and 3,1 for spiking level by PE-I9014
SP	6				
SP	21				
VT	3			yes	
VT	17				
VT	19			yes	

5.1.4 ELISA: Soyprotein

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	%		Test-Kit + Manufacturer
BC	4	09.03.21	positive	0,3458	negative	<0.05	positive	0,5147	0,05	0,05	50	Soy trypsin inhibitor	BioCheck ELISA Soya-Check
MI-II	6		positive	21	negative	<1,25	positive	17	1,25	1,25		Soyprotein	Morinaga Soyprotein ELISA Kit II
MI-II	12	14.04.21	-	27	-	<0,3	-	33	0,3	1,25		Soyprotein	Morinaga Soya ELISA Kit II
RS-F	1	03.03.21	positive	>20	negative	<2,5	positive	>20		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	5		positive	> 20	negative	< 2,5	positive	> 20		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	9		Detected	18,10	Not detected	<2,5	Detected	11,82		2,5		soya protein	r7102
RS-F	10	06.04.	positive	17	negative	<2,5	-		0,24	2,5	63,1	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	11	03.03.21	positive		negative		positive		2,5	2,5		Please select!	andere: bitte eingeben!
RS-F	14	25.02.21	positive	10,1	negative	No detectado,<2,5	positive	12,7		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	16	17.03.21	positive	35,73	negative	<2,5	positive	50,39	0,24	2,5	23,1	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	18a	04.03.21	positive	31,03	negative	<2.5	positive	30,33	2,5	2,5	24,54	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	19a	03.05.21	positive	27	negative	<2.5	positive	41	2,5	2,5		Soyprotein	RIDASCREEN FAST Soya, r-Biopharm
SP	13	23.03.21	positive	11	negative	<2	positive	26	0.2	2		Soyprotein	SENSISpec Total Soy Protein
SP	21		positive		negative		positive		0,016	0,04	30	Please select!	SensiSpec ELISA Soy, Eurofins
VT	2	15.03.21	positive	25	negative	< 2,5	positive	26		2,5		Soyflour	Veratox Soy Allergen, Neogen
VT	3	09.03.21	-	16,6	-	n.n.	-	17,9	0,5	1,3	40	Soyprotein	Veratox Soja Allergen Neogen
VT	7		positive	31	negative	0	positive	44				Soyprotein	andere: NEOGEN Veratox Soy Allergen!
VT	8	24.03.21	positive	34	negative	0,35	positive	37	0,96	2,5		Soyflour	Verotox Soy Allergen, Neogen 8410
VT	17	16/4	-	25,5	-	<2.5	-	19,6S		2,5		food	Selection Soy-Kits: Neogen
VT	18b	26.02.21	positive	25,75	negative	<2.5	positive	31	2,5	2,5	20,23	Soyflour	Veratox Soy Allergen, Neogen
VT	19b	23.04.21	positive	29	negative	<2.5	positive	28	2,5	2,5		Soyflour	Veratox Soy Allergen, Neogen

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

Continuation ELISA Soja:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BC	4		0.5g sample/10ml kit extraction buffer at 60C/15mins	yes	
MI-II	6		As Per Kit Instructions	yes	
MI-II	12			yes	
RS-F	1	Soy protein		yes	
RS-F	5			yes	
RS-F	9				
RS-F	10			yes	
RS-F	11			yes	Ridascreen FAST Soya R7102
RS-F	14			yes	
RS-F	16			no	
RS-F	18a	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	19a		Detection of Soya even after processing	yes	Sample A+B: Protocol for spices
SP	13				
SP	21				
VT	2		2g in 125 mL PBS (supplied by the kit) / 15 minutes / 60°C	yes	
VT	3			no	
VT	7	Soy Protein ELISA	15 min / 60°C		
VT	8		Extraction solution (10 mM PBS, pH 7,4) / 60°C / 15 min		Single results sample A: 35,0 mg/kg, 33,0 mg/kg Single results Probe B: 0,5 mg/kg, 0,2 mg/kg Single results Dotierungsprobe: 37 mg/kg, 37 mg/kg
VT	17				
VT	18b	As Per Kit Instructions	As Per Kit Instructions	Yes	
VT	19b		Detection of soyflour not processed	yes	Detection of soyflour not processed

5.1.5 PCR: Milk

Meth. Abr.	Evaluation no.	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 /protein	Test-Kit+Manufacturer
div	19	04.05.21	positive		negative		positive					DNA-Cow	

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
div	19			yes	

5.1.6 PCR: Soya

Meth. Abr.	Evaluation no.	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 /protein	Test-Kit+Manufacturer
GR	20		negative		negative		positive					Please select!	SPECIALfinder Assay, real time PCR, Generon
GR	22		negative		negative		positive					Please select!	SPECIALfinder Assay, real time PCR, Generon
SFA	5		positive		negative		positive		0,4			Soy-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21		positive		negative		positive		0,4			Please select!	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	18	09.03.21	positive	20,92	negative	<1	positive	34,61	1	1	23,73	Soybean	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	11	25.02.21	positive		negative		positive		<0,4			Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	6		positive		negative		-		5			Soy-DNA	interne Method
div	19	29.04.21	positive		negative		positive					Soy-DNA	Auswahl PCR-Methoden

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
GR	20			no	
GR	22			no	
SFA	5			yes	
SFA	21				
SFA-ID	18	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA-Q	11			yes	
div	6		CTAB / Proteinase K / RNase A / Promega Maxwell / Realtime PCR / 45 Cycles	yes	
div	19	Lectin-Gene		yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptAL01 Sample A

Weight whole sample	2,41	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	61	24,4
2	5,00	66	26,4
3	5,02	64	25,5
4	5,02	56	22,3
5	5,03	56	22,3
6	5,01	50	20,0
7	5,02	57	22,7
8	5,03	62	24,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,0	Particles
Standard deviation	5,25	Particles
χ^2 (CHI-Quadrat)	3,27	
Probability	**86**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	23,5	mg/kg
Standard deviation	2,09	mg/kg
rel. Standard deviaton	8,89	%
Horwitz standard deviation	9,95	%
HorRat-value	**0,89**	
Recovery rate	105	%

Microtracer Homogeneity Test
DLA ptAL01 Spiking Level Sample

Weight whole sample	1,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	71	28,3
2	5,02	70	27,9
3	5,04	69	27,4
4	5,04	67	26,6
5	5,01	61	24,4
6	5,01	58	23,2
7	4,97	60	24,1
8	4,98	69	27,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,6	Particles
Standard deviation	5,01	Particles
χ^2 (CHI-Quadrat)	2,67	
Probability	**91**	%
Recovery rate	90	%

Normal distribution		
Number of samples	8	
Mean	26,2	mg/kg
Standard deviation	2,00	mg/kg
rel. Standard deviaton	7,63	%
Horwitz standard deviation	9,79	%
HorRat-value	**0,78**	
Recovery rate	90	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL01 - 2021***
PT name	***Allergens I: Milk (Casein) and Soya in Sauce Powder with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *Sauce Powder / ingredients: Starch (potato), salt, vegetables (onions, carrots, leeks, parsnips, potatoes, celery, garlic, parsley), corn starch, raw cane sugar, herbs, spices, sunflower oil, flavor enhancer (sodium gluatamate, disodium inosinate), anti-caking agent: silicon dioxide; natural fenugreek flavor, other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A, B + Spiking Level Sample:* *room temperature (PT period), cooled 2 - 10°C (long term)*
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***
Last Deadline	***the latest April 16th 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

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

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
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 - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for

Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)

24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 00.00-169 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Erdnuss in Lebensmitteln mittels real-time PCR (2019) [Foodstuffs, detection and determination of peanut in foods by real-time PCR]
32. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
33. ASU §64 LFGB L 16.01-9 Untersuchung von Lebensmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016) [Foodstuffs, determination of soya (Glycine max) in cereal flour by real-time PCR]
34. ASU §64 LFGB L 08.00-59 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
35. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2017) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]
36. Allergen Data Collection - Update (2002): Cow's Milk (Bos domesticus), Besler M., Eigenmann P., Schwartz R., Internet Symposium on Food Allergens 4(1): 19-106, http://www.food-allergens.de
37. Allergen Data Collection - Update (2002): Soybean (Glycine max), Besler M., Helm R.M., Ogawa T., Internet Symposium on Food Allergens 2(Suppl.3): 1-35 (2000) http://www.food-allergens.de