Evaluation Report
proficiency test

DLA 03/2019

Allergens III:

β-Lactoglobulin, Casein and Gluten

in Infant Food

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

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EP-Nummer *PT-Number*	DLA 03/2019
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (14 August 2019) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 14 August 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a customary infant food "cereal pap" from 6th month on (labeled as dairy-free and gluten-free). The basic composition of samples A and B was the same (see table 1). After crushing and sieving (mesh <1,5 mm) the basic mixture was homogenized.
Afterwards the **spiked sample B** was produced as follows:
The spiking materials containing the allergenic ingredients skimmed milk powder, whey powder and wheat flour were crushed and sieved by a centrifugal mill (mesh <250 µm or <500 µm), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in up to 3 additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Organic-Cereal-Pap, infant pap after the 6th month Ingredients: Rice flour (70%), corn flour (20%), millet wholemeal flour (10%), thiamine Nutrients per 100 g: Fat 2,8 g, Carbohydrates 80 g, Protein 8,7 g	100 g/100 g	99,8 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,8 g/100 g
Milk component 1: skimmed milk powder mixture (9 products from Europe, USA) - as skimmed milk powder* - thereof 33,0% total protein** - thereof Casein*** - thereof β-Lactoglobulin***	-	1054 mg/kg 348 mg/kg 278 mg/kg 34,8 mg/kg	992 mg/kg 327 mg/kg 262 mg/kg 32,7 mg/kg
Milk component 2: whey powder mixture (4 products from Germany) - as whey powder * - thereof 15,9% total protein** - *thereof β-Lactoglobulin****		343 mg/kg 54,5 mg/kg 27,2 mg/kg	353 mg/kg 56,2 mg/kg 28,1 mg/kg
Sum of milk components - thereof total protein** - thereof Casein*** - *thereof β-Lactoglobulin****		*1400 mg/kg* 403 mg/kg *278 mg/kg* *62,0 mg/kg*	*1345 mg/kg* 383 mg/kg *262 mg/kg* *60,8 mg/kg*
Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) - as wheat flour* - thereof 10,1% total protein** - thereof gluten***	-	367 mg/kg 37,1 mg/kg 31,9 mg/kg	416 mg/kg 42,0 mg/kg 36,2 mg/kg
further Ingredients: *Maltodextrin, sodium sulfate and silicon dioxide*	-	<0,2 g/100 g	<0,2 g/100 g

* Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw materials (total nitrogen according to Kjeldahl with F=6,38 for milk protein and F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 80% casein and 10% β-latcoglobulin in total milk protein [31]; approx. 50% approx. β-Lactoglobulin in whey powder [31]; 8,7% gluten in wheat flour [32, 33, 34])

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 B and the spiking level sample showed a probability of 99% and 95%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17].
This gave a HorRat value of 0,6 and 0,6 respectively. Aufgrund der ausreichenden Wahrscheinlichkeit wurde der HorRat-Wert für Probe B akzeptiert. The HorRat value of sample B was accepted because of the sufficient probability. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis. The sample weights were made with a deviation of ± 10% from recommended sample weight of the test kit instructions and not communicated to the laboratories. After transmission of analysis results by the laboratories, the valid results were calculated on the basis of the exact weightings by DLA and the statistical calculation was carried out according to ISO 13528:2015 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 B by all ELISA tests for milk / milkproteins (Immunolab, Veratox and AgraQuant) and gluten (Immunolab, Veratox and AgraQuant), respectively (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

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

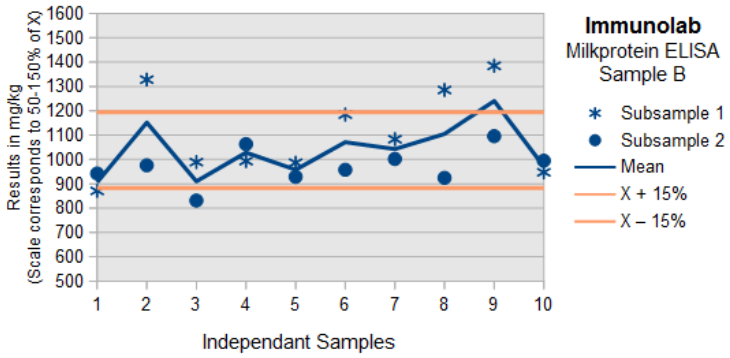
ELISA-Tests: Homogenität Milch / Homogeneity Milk

Immunolab Milkprotein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Milkprotein 1040 ± 78 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	871	942	907
2	1328	976	1152
3	989	831	910
4	993	1063	1028
5	986	929	958
6	1185	958	1071
7	1083	1002	1043
8	1286	924	1105
9	1385	1096	1240
10	947	995	971

General average X	1038	
SD of sample means Sx	107,6	10,4%
SD w ithin-samples Sw	104,4	10,1%
SD betw een-samples Ss	78,2	7,5%

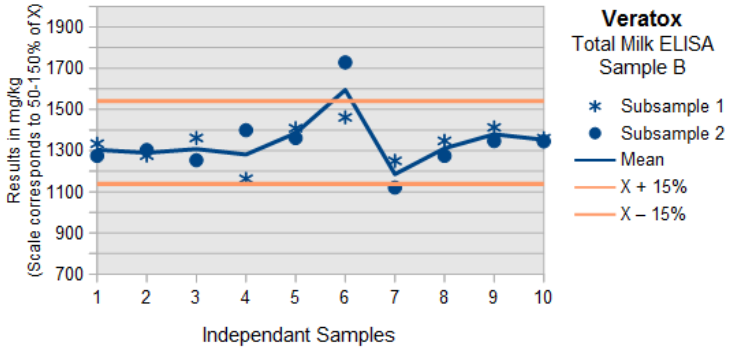


Veratox Total Milk ELISA

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Non-fat dry milk 1340 ± 96 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	1334	1274	1304
2	1276	1303	1289
3	1362	1253	1307
4	1163	1399	1281
5	1409	1361	1385
6	1461	1728	1595
7	1250	1120	1185
8	1347	1274	1311
9	1413	1346	1379
10	1360	1345	1353

General average X	1339	
SD of sample means Sx	106	7,9%
SD w ithin-samples Sw	65,5	4,9%
SD betw een-samples Ss	95,7	7,1%



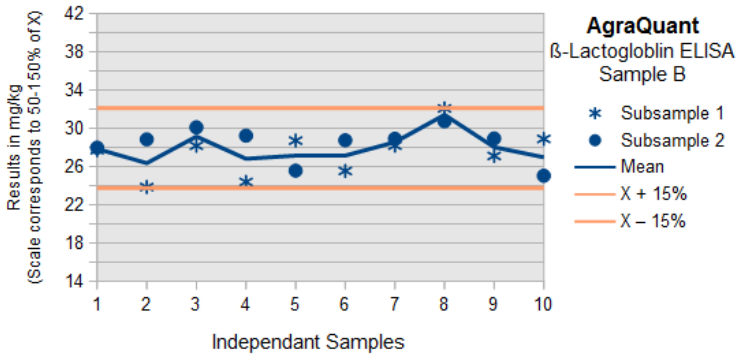
ELISA-Tests: Homogenität β-Lactoglobulin / Homogeneity β-Lactoglobulin

AgraQuant β-Lactoglobulin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: β-Lactoglobulin 27,9 ± 1,0 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	27,7	28,0	27,8
2	23,8	28,8	26,3
3	28,2	30,1	29,1
4	24,4	29,2	26,8
5	28,7	25,6	27,1
6	25,5	28,7	27,1
7	28,2	28,9	28,5
8	32,1	30,7	31,4
9	27,1	28,9	28,0
10	28,9	25,0	27,0

General average X	27,9	
SD of sample means Sx	1,49	5,3%
SD w ithin-samples Sw	1,52	5,5%
SD betw een-samples Ss	1,02	3,7%



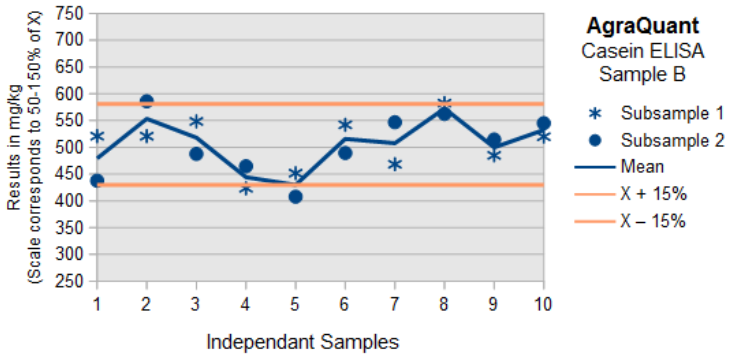
ELISA-Tests: Homogenität Casein / Homogeneity Casein

AgraQuant Casein ELISA

Sample weights: 0,50 g (0,45 – 0,55 g)
Number of replicates: 2
Overall result: Casein 505 ± 40 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	521	438	479
2	521	586	553
3	548	488	518
4	424	465	444
5	452	408	430
6	542	489	516
7	468	547	508
8	583	562	572
9	485	515	500
10	520	545	532

General average X	505	
SD of sample means Sx	44,7	8,8%
SD w ithin-samples Sw	27,1	5,4%
SD betw een-samples Ss	40,4	8,0%



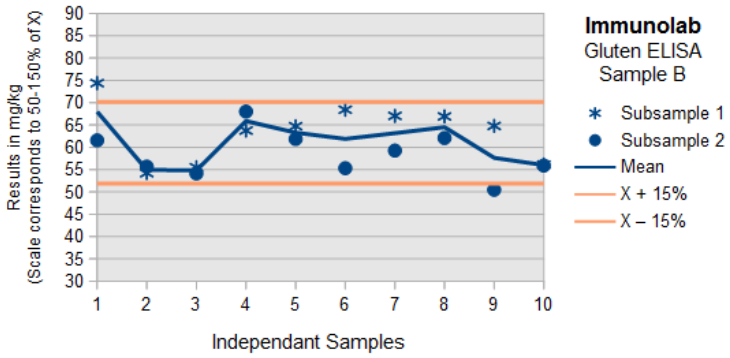
ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 61,0 ± 3,8 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	74,4	61,6	68,0
2	54,2	55,7	54,9
3	55,5	54,1	54,8
4	63,8	68,0	65,9
5	64,7	61,9	63,3
6	68,4	55,3	61,9
7	67,0	59,3	63,2
8	66,9	62,1	64,5
9	64,8	50,5	57,6
10	56,1	55,9	56,0

General average X	61,0	
SD of sample means Sx	4,79	7,8%
SD w ithin-samples Sw	4,05	6,6%
SD betw een-samples Ss	3,84	6,3%

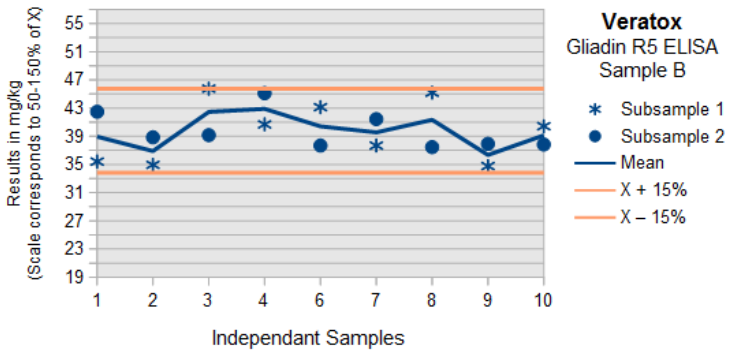


Neogen Veratox ELISA Gluten R5

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Gluten 39,8 ± 1,3 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	35,4	42,5	38,9
2	34,9	38,8	36,9
3	45,8	39,2	42,5
4	40,7	45,1	42,9
6	43,1	37,7	40,4
7	37,7	41,4	39,6
8	45,2	37,5	41,3
9	34,8	37,9	36,3
10	40,4	37,8	39,1

General average X	39,8	
SD of sample means Sx	2,26	5,7%
SD w ithin-samples Sw	2,63	6,6%
SD betw een-samples Ss	1,29	3,2%

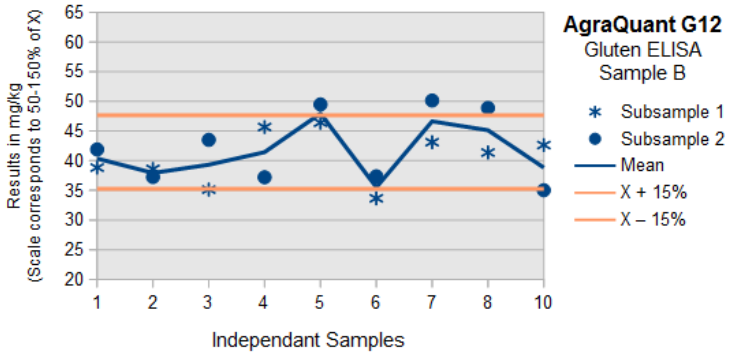


Romerlabs AgraQuant Gluten G12

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Gluten 41,5 ± 3,6 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	38,8	41,9	40,4
2	38,6	37,2	37,9
3	35,1	43,5	39,3
4	45,7	37,2	41,4
5	46,4	49,5	47,9
6	33,7	37,4	35,5
7	43,1	50,1	46,6
8	41,4	48,9	45,1
10	42,6	35,0	38,8

General average X	41,5	
SD of sample means Sx	4,22	10,2%
SD w ithin-samples Sw	3,07	7,4%
SD betw een-samples Ss	3,62	8,7%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,16 (22,5°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 11th week of 2019. The testing method was optional. The tests should be finished at 26th April 2019 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters β-Lactoglobulin, Casein and Gluten in the partly higher range of mg/kg in the matrix of Infant Food (semolina powder with rice, maize and sorghum). 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.

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, 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.

Of 27 participants, 25 participants submitted their results on time. 2 participants did not submit any results.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 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 saus-age (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled saus-age (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59
Wheat + Rye	Boiled saus-age (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

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

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

β-Lactoglobulin-specific ELISA results given as **total milk protein** were converted to **β-lactoglobulin** using contents from the literature [36] (approx. 10 % in total milk protein, see S.5) (Morinaga ELISA Kit II).

Casein-specific ELISA results given as **skimmed milk powder** were converted to **casein**. For this the information supplied in the manufacturer's test kit instructions for the content of casein in skimmed milk powder were taken (ELISA-Systems Test-Kit Manual: 25,6%).
Casein-specific ELISA results given as **total milk protein** were converted to **casein** using contents from the literature [36] (approx. 80 % in total milk protein, see S.5) (Morinaga ELISA Kit II).

Milk protein-specific ELISA results given as **skimmed milk powder** were converted to **total milk protein** using the analysed protein content of the raw material (see page 5; Neogen Veratox, Ridascreen Fast).

In the present PT all gluten ELISA results were submitted as gluten, therefore no conversion was necessary.

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

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

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

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

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

Characteristics	**All Results** *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ i}}$
Number of results		
Number of outliers		
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 $U_{(Xpt)}$		
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 β-Lactoglobulin

4.1.1 ELISA Results: β-Lactoglobulin

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
1	negative	<LOD	positive	18,7	2/2 (100%)	AQ	
18	negative	<LOD	positive	18,0	2/2 (100%)	AQ	
20	negative	< LOD	positive	16,0	2/2 (100%)	AQ	
4	negative	<0,1	positive	>1	2/2 (100%)	ES	
13	negative	<0,10	positive	98,0	2/2 (100%)	ES	
14	negative	<LOD	positive	137	2/2 (100%)	ES	
12	negative	<0,01	positive	207	2/2 (100%)	IL	
25	negative	0	positive	14,2	2/2 (100%)	IL	
16	negative		positive	32,5	2/2 (100%)	MI-II	Result converted °
17	negative	<0,031	positive	32,0	2/2 (100%)	MI-II	
10	negative	<2,63	positive	36,4	2/2 (100%)	RS	
2	negative		positive	10,0	2/2 (100%)	RS-F	
5	negative	0,07	positive	4,56	2/2 (100%)	RS-F	
8	negative	<1,5	positive	36,0	2/2 (100%)	RS-F	
9	negative		positive	29,1	2/2 (100%)	RS-F	
11	negative		positive		2/2 (100%)	RS-F	
15	negative		positive	> 4,5	2/2 (100%)	RS-F	
21	negative	<0,2	positive	>4,5	2/2 (100%)	RS-F	
24	negative	<0,167	positive	18,9	2/2 (100%)	RS-F	

° calculation see p. 20

	Sample A		Sample B	
Number positive	0		19	
Number negative	19		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

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

Quantitative valuation of ELISA-results: Sample B

Evaluation number	β-Lacto-globulin	z'-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]		informative		
1	18,7	-0,69		AQ	
18	18,0	-0,78		AQ	
20	16,0	-1,0		AQ	
4	>1			ES	
13	98,0	9,8		ES	
14	137			ES	Result excluded
12	207			IL	Result excluded
25	14,2	-1,3		IL	
16	32,5	1,1		MI-II	Result converted °
17	32,0	1,1		MI-II	
10	36,4	1,7		RS	
2	10,0	-1,8	-2,0	RS-F	
5	4,56	-2,5	-3,1	RS-F	
8	36,0	1,6	3,3	RS-F	
9	29,1	0,69	1,9	RS-F	
11				RS-F	
15	> 4,5			RS-F	
21	>4,5			RS-F	
24	18,9	-0,66	-0,17	RS-F	

° calculation see p. 20

Methoden:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

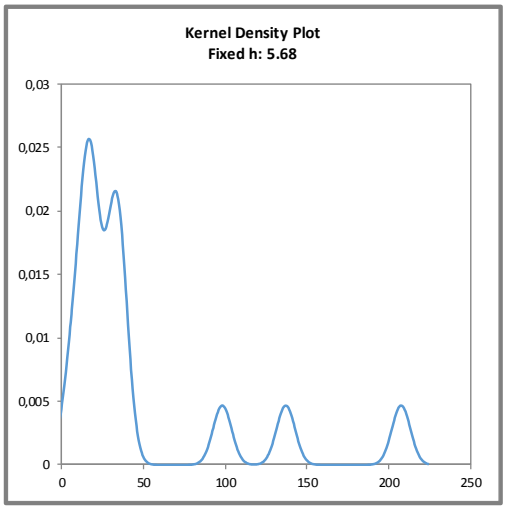


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a distribution of results with two maxima (results of 3 methods each) and three smaler peaks at > 60 mg/kg (methods ES and IL) due to some single results above the target range.

Characteristics: Quantitative evaluation ELISA β-Lactoglobulin

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** [mg/kg]
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS-F}}$
*Number of results**	13	5
Number of outliers	2	0
Mean	28,0	19,7
Median	18,9	18,9
Robust Mean (X_{pt})	**23,9**	**19,7**
Robust standard deviation (S*)	**13,4**	**14,8**
Target range:		
Target standard deviation $\sigma_{pt'}$ and σ_{pt}	**7,57**	**4,93**
lower limit of target range	**8,73**	**9,85**
upper limit of target range	**39,0**	**29,6**
Quotient S/$\sigma_{pt'}$ and S*/σ_{pt}*	*1,8*	*3,0*
Standard uncertainty $U_{(Xpt)}$	*4,66*	*8,25*
Results in the target range	*11*	*3*
Percent in the target range	*85*	*60*

*without results No. 12 and 14 (as outlier excluded)

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences, after excluding two results.
The evaluation of all methods showed an increased variability of results, with a quotient S/σ_{pt} of 2,3. Therefore the evaluation of all methods was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was then below 2,0.*
The robust standard deviation is above the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods, because there were only a few results for some methods.
The evaluation of the method RS-F showed with few available results a high variability of results. Therefore the evaluation is given for information only.
The robust means of the evaluations were 39% and 32% of the spiking level of β-lactoglobulin to sample B below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of β-lactoglobulin" p.30).

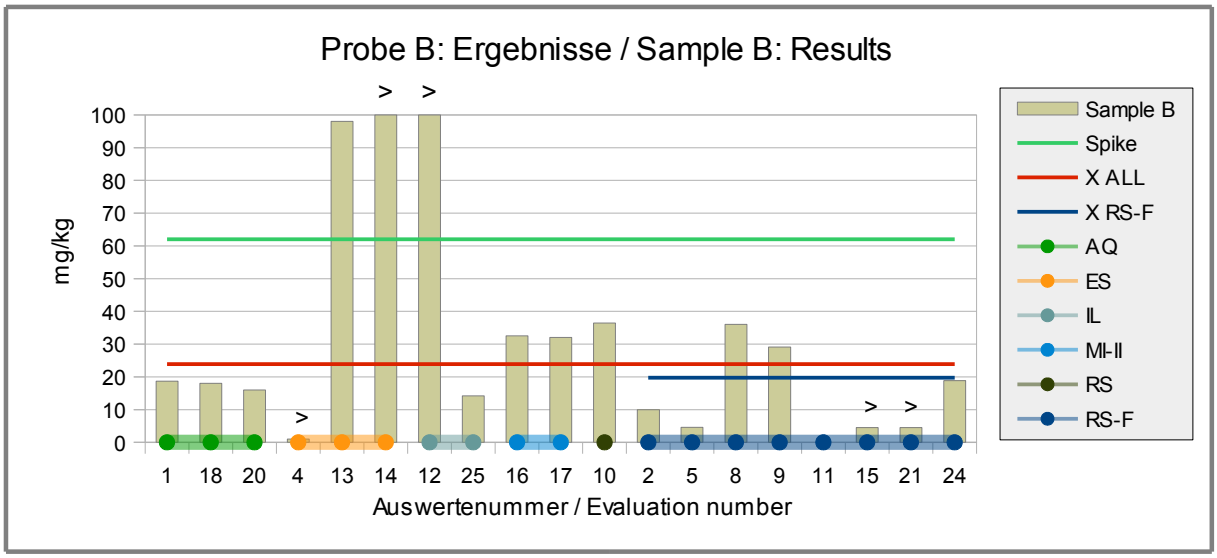


Abb./Fig. 2: ELISA Results β-Lactoglobulin
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

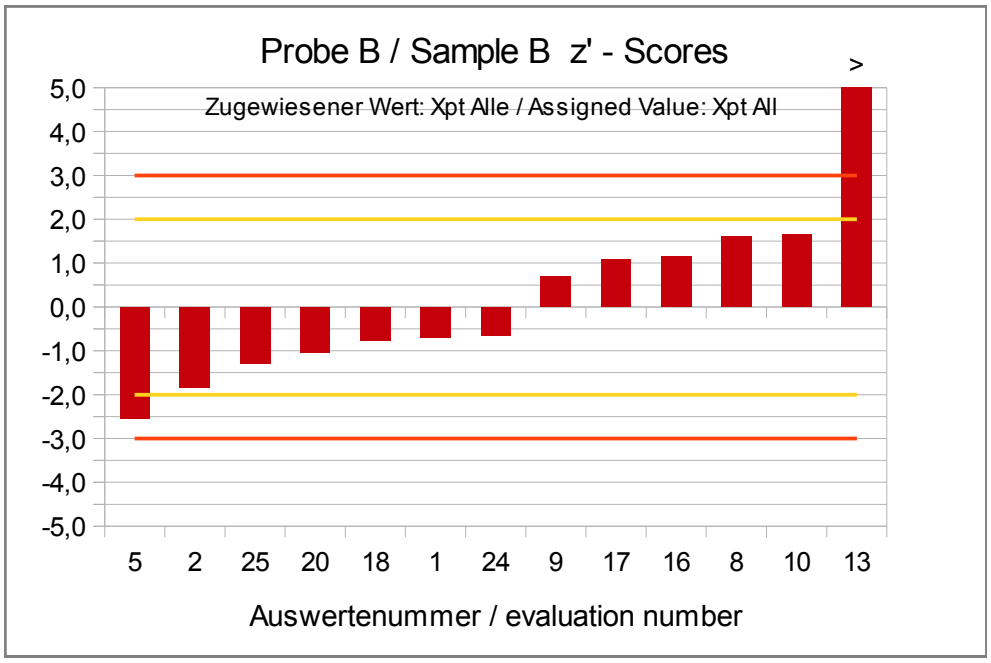


Abb./Fig. 3:
z'-Scores (ELISA Results β-Lactoglobulin)
Assigned value robust mean of all results

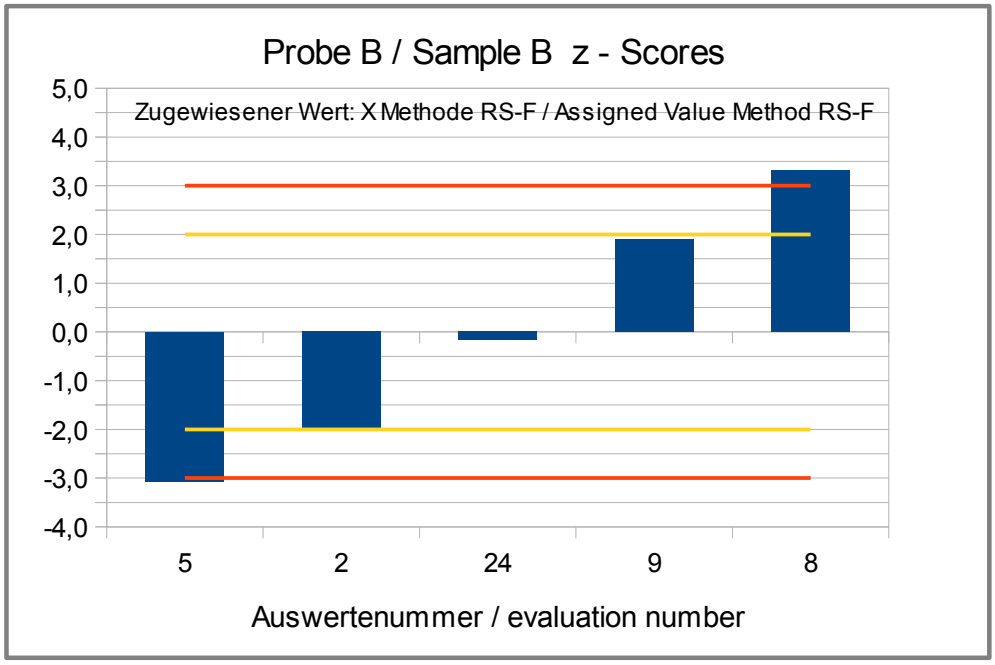


Abb./Fig. 4:
z-Scores for information (ELISA Results β-Lactoglobulin)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	β-Lactoglo-bulin	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
1	18,8	-0,94	AQ	
18	16,0	-1,4	AQ	
20	16,1	-1,4	AQ	
4	>1		ES	
13	93,0	11	ES	
14			ES	
12	14,5	-1,6	IL	
25	12,9	-1,9	IL	
16	31,0	1,1	MI-II	Result converted °
17	31,0	1,1	MI-II	
10	28,9	0,72	RS	
2			RS-F	
5	4,53	-3,3	RS-F	
8	36,7	2,0	RS-F	
9	33,8	1,5	RS-F	
11			RS-F	
15	> 4,5		RS-F	
21	>4,5		RS-F	
24	29,7	0,85	RS-F	

° calculation see p. 20

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

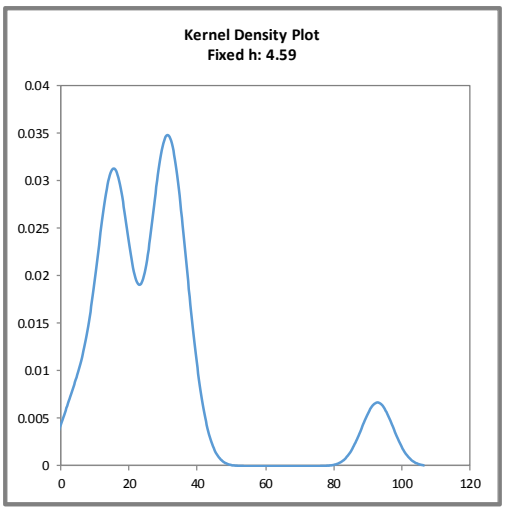


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a distribution of results with two maxima (results of 3 methods each) and a additional peak at approx. 90 mg/kg due to a single result (method ES).

Characteristics: Quantitative evaluation ELISA β-Lactoglobulin

Spiking Level Sample

Statistic Data	**All Results [mg/kg]**
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	13
Number of outliers	-
Mean	28,2
Median	28,9
Robust Mean (X_{pt})	**24,5**
Robust standard deviation (S*)	**12,7**
Target range:	
Target standard deviation σ_{pt}	**6,12**
lower limit of target range	**12,2**
upper limit of target range	**36,7**
Quotient S/σ_{pt}*	*2,1*
Standard uncertainty $U_{(Xpt)}$	*4,40*
Results in the target range	*11*
Percent in the target range	*85*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (two maxima of several methods, one high single value).

The evaluation of all methods showed a slightly increased variability of results, with a quotient S/σpt of 2,1.*
The robust standard deviation is above the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust mean of the evaluation was 40% of the spiking level of β-lactoglobulin to spiking level sample below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of β-lactoglobulin" p.30).

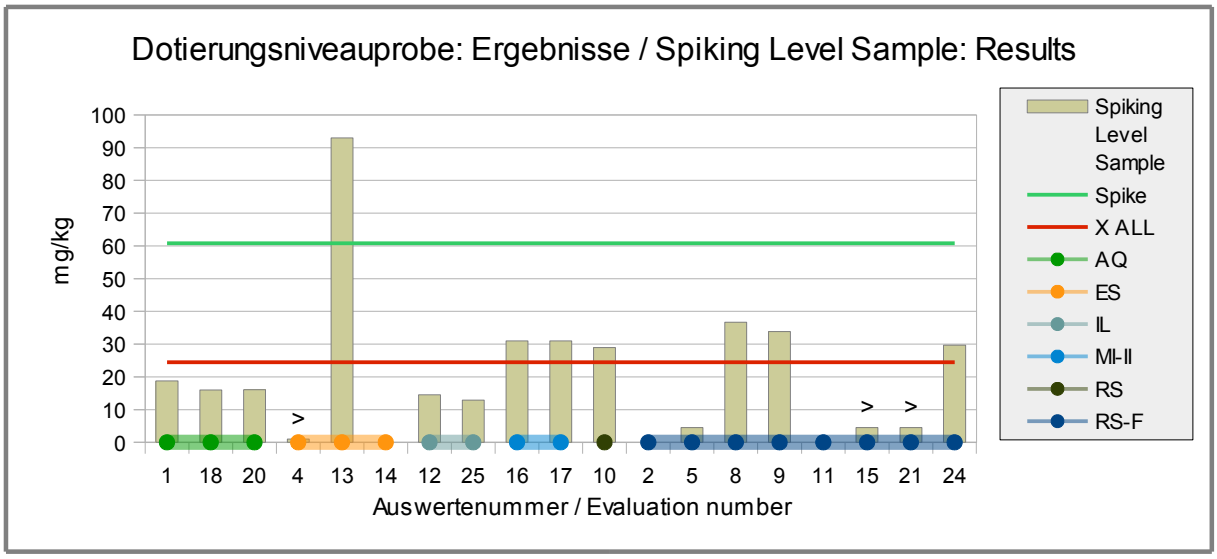


Abb./Fig. 6: ELISA Results β-Lactoglobulin
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

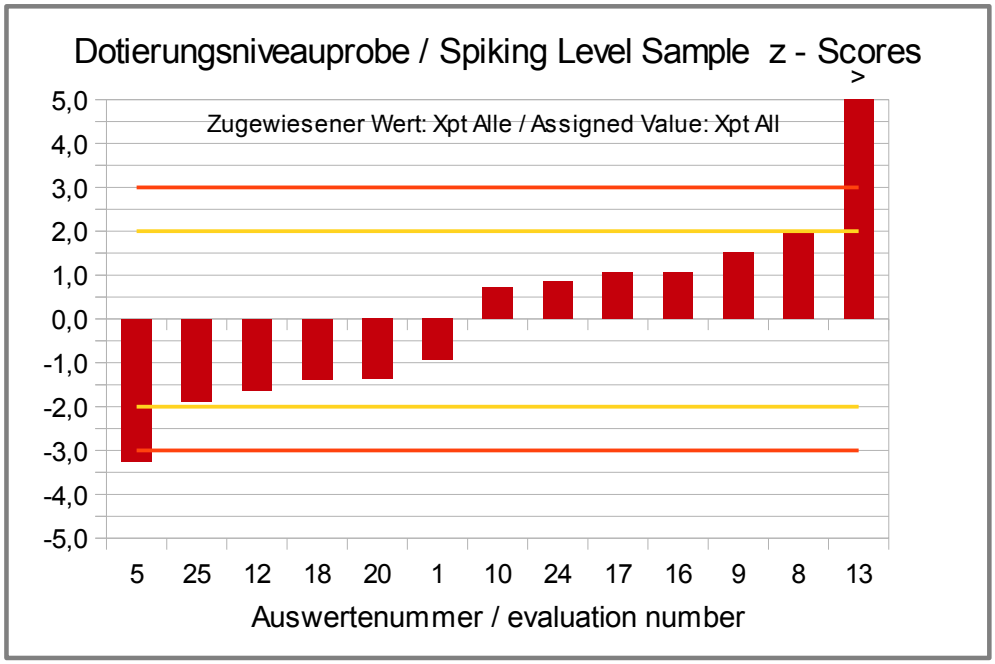


Abb./Fig. 7:
z'-Scores (ELISA Results β-Lactoglobulin)
Assigned value robust mean of all results

Recovery Rates ELISA for β-Lactoglobulin: Spiking level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	18,8	31	18,7	30	AQ	
18	16,0	26	18,0	29	AQ	
20	16,1	26	16,0	26	AQ	
4	>1		>1		ES	
13	93,0	153	98	158	ES	
14			137	221	ES	
12	14,5	24	207	334	IL	
25	12,9	21	14,2	23	IL	
16	31,0	**51**	32,5	**52**	MI-II	Result converted °
17	31,0	**51**	32,0	**52**	MI-II	
10	28,9	48	36,4	**59**	RS	
2			10,0	16	RS-F	
5	4,53	7,5	4,56	7,4	RS-F	
8	36,7	**60**	36,0	**58**	RS-F	
9	33,8	**56**	29,1	47	RS-F	
11					RS-F	
15	> 4,5		> 4,5		RS-F	
21	>4,5		>4,5		RS-F	
24	29,7	49	18,9	**30**	RS-F	

° calculation see p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**4**	Number in RA	**4**
Percent in RA	**31**	Percent in RA	**27**

* Recovery rate 100% relative size: β-Lactoglobulin, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For the spiking level sample 31% (4) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 27% (4) of the recovery rates were within the range of acceptance.

4.2 Proficiency Test Casein / Milk protein

4.2.1 ELISA Results: Casein

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
1	negative	<LOD	positive	1475	2/2 (100%)	AQ	
8	negative	<1,5	positive	332	2/2 (100%)	AQ	
9	negative		positive	424	2/2 (100%)	AQ	
13	negative	<0,20	positive	410	2/2 (100%)	AQ	
18	negative	<LOD	positive	498	2/2 (100%)	AQ	
19	negative	< 0,2	positive	425	2/2 (100%)	AQ	
20	negative	< LOD	positive	436	2/2 (100%)	AQ	
6	negative	0	positive	198	2/2 (100%)	BF	
4	negative	<0,256	positive	>2,56	2/2 (100%)	ES	Result converted °
14	negative	<LOD	positive	47,7	2/2 (100%)	ES	
12	negative	<0,2	positive	163	2/2 (100%)	IL	
25	negative	0	positive	477	2/2 (100%)	IL	
16	negative		positive	302	2/2 (100%)	MI-II	Result converted °
17	negative	<0,25	positive	340	2/2 (100%)	MI-II	
2	negative		positive	7,90	2/2 (100%)	RS-F	
5	negative	0,49	positive	77,4	2/2 (100%)	RS-F	
11	negative		positive	340	2/2 (100%)	RS-F	
15	negative		positive	>67,5	2/2 (100%)	RS-F	
21	negative	<3,0	positive	640	2/2 (100%)	RS-F	
24	negative	<2,5	positive	200	2/2 (100%)	RS-F	

° calculation see p. 20

	Sample A		Sample B	
Number positive	0		20	
Number negative	20		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
The consensus value is in qualitative agreement with the spiking of sample B.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Casein	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
1	1475	14	9,4	AQ	
8	332	0,00	-1,0	AQ	
9	424	1,1	-0,16	AQ	
13	410	0,94	-0,29	AQ	
18	498	2,0	0,51	AQ	
19	425	1,1	-0,15	AQ	
20	436	1,3	-0,05	AQ	
6	198	-1,6		BF	
4	>2,56			ES	Result converted °
14	47,7	-3,4		ES	
12	163	-2,0		IL	
25	477	1,7		IL	
16	302	-0,35		MI-II	Result converted °
17	340	0,10		MI-II	
2	7,90	-3,9		RS-F	
5	77,4	-3,1		RS-F	
11	340	0,10		RS-F	
15	>67,5			RS-F	
21	640	3,7		RS-F	
24	200	-1,6		RS-F	

° calculation see p. 20

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm

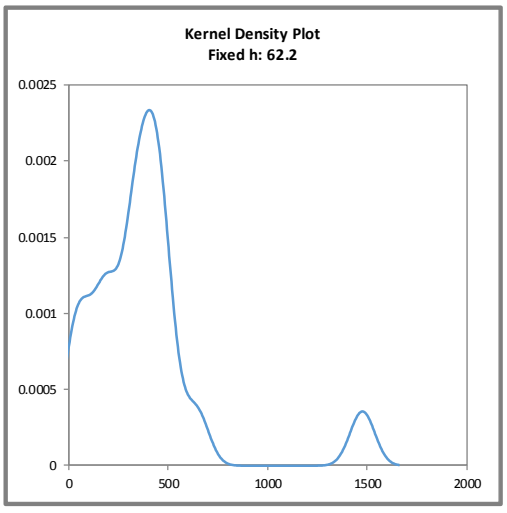


Abb. / Fig. 8:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a broad shoulder at <350 mg/kg, a smaller shoulder at about 640 mg/kg and a side peak at approx. 1500 mg/kg, due to a single value above the target range.

Characteristics: Quantitative evaluation ELISA Casein

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	18	7
Number of outliers	–	–
Mean	377	571
Median	340	425
Robust Mean (X_{pt})	**332**	**442**
Robust standard deviation (S*)	**211**	**83,5**
Target range:		
Target standard deviation σ_{pt}	**83,0**	**110**
lower limit of target range	**166**	**221**
upper limit of target range	**498**	**662**
Quotient S/σ_{pt}*	*2,5*	*0,76*
Standard uncertainty U(X_{pt})	*62,2*	*39,4*
Results in the target range	*14*	*6*
Percent in the target range	*78*	*86*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

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

The evaluation of all methods showed an increased variability of results, with a quotient S/σpt of 2,5. The evaluation of the results of method AQ showed a low variability of results. The quotient S*/σpt was below 1,0.*
The robust standard deviation is increased for the the evaluation across the methods and is for the method AQ in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 119% and 159% of the spiking level of casein to sample B and thus within or just above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Casein" p.40).

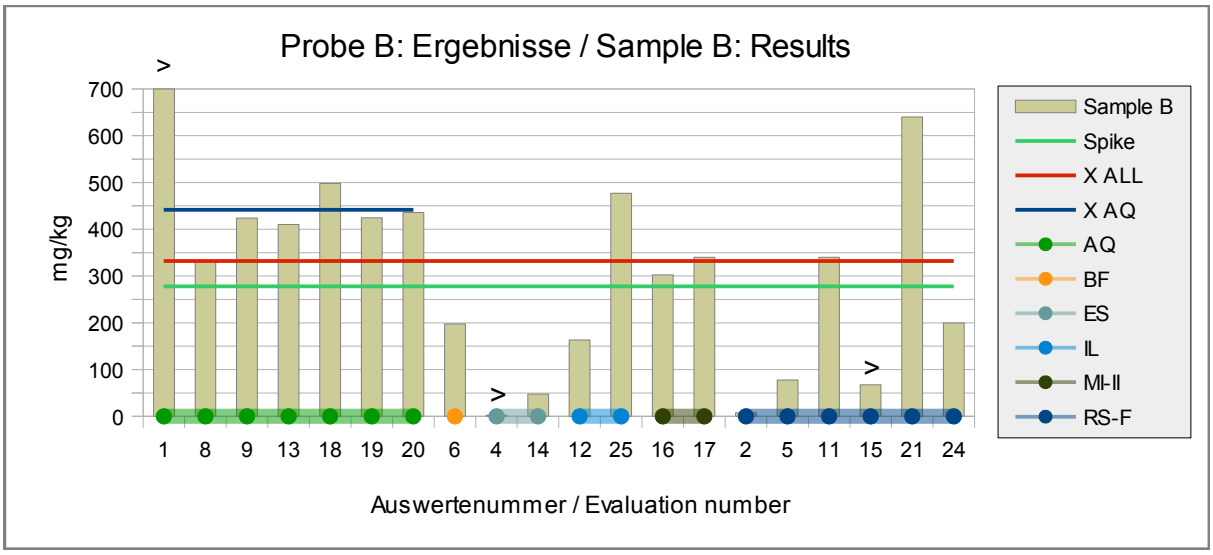


Abb./Fig. 9: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

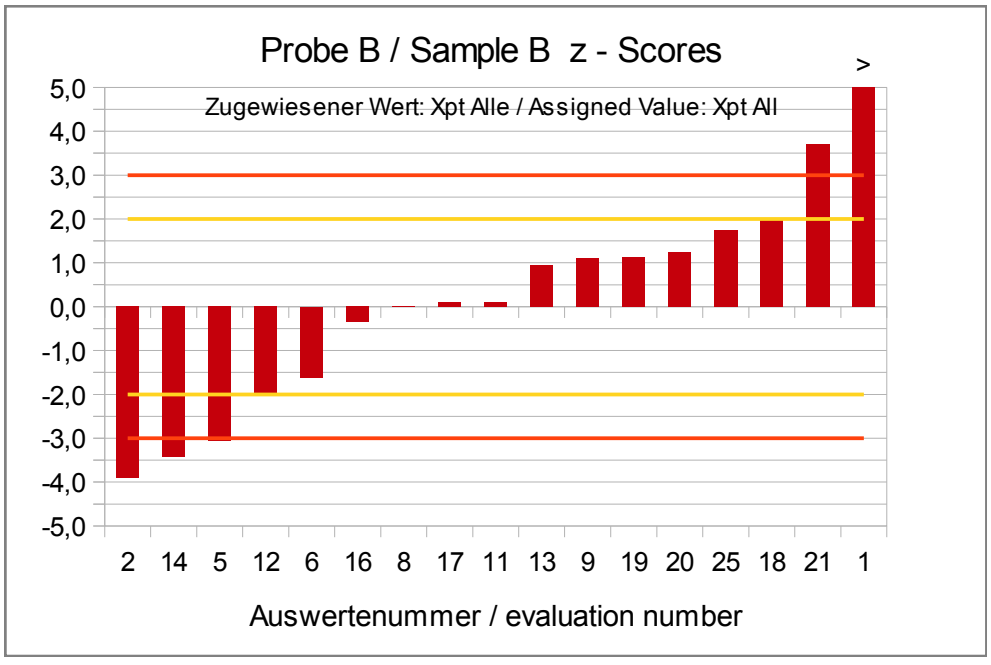


Abb./Fig. 10:
z-Scores (ELISA Results Casein)
Assigned value robust mean of all results

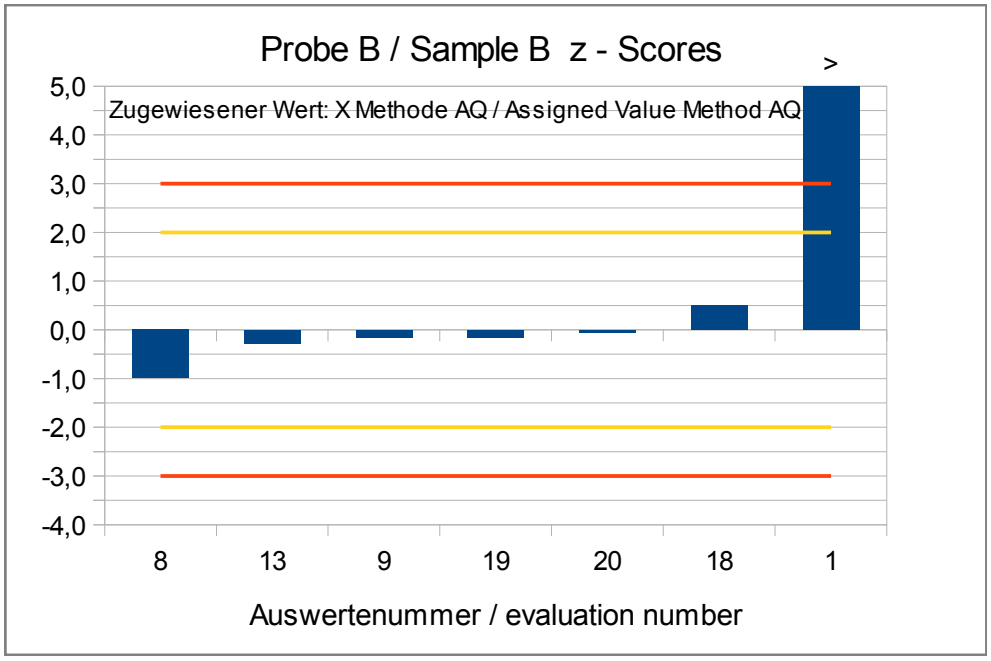


Abb./Fig. 11:
z-Scores (ELISA Results Casein)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Casein	z-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
1	1950		16,6	AQ	Result excluded for Xpt_{ALL}
8	144	-2,1	-2,5	AQ	
9	393	1,2	0,14	AQ	
13	470	2,3	1,0	AQ	
18	311	0,14	-0,72	AQ	
19	356	0,74	-0,24	AQ	
20	346	0,61	-0,35	AQ	
6	117	-2,4		BF	
4	>2,56			ES	Result converted °
14		-4,0		ES	
12	16,5			IL	Result excluded for Xpt_{ALL}
25	277	-0,31		IL	
16	268	-0,44		MI-II	Result converted °
17	250	-0,67		MI-II	
2		-4,0		RS-F	
5	68,3	-3,1		RS-F	
11	370	0,93		RS-F	
15	>67,5			RS-F	
21	610	4,1		RS-F	
24	304	0,05		RS-F	

° calculation see p. 20

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm

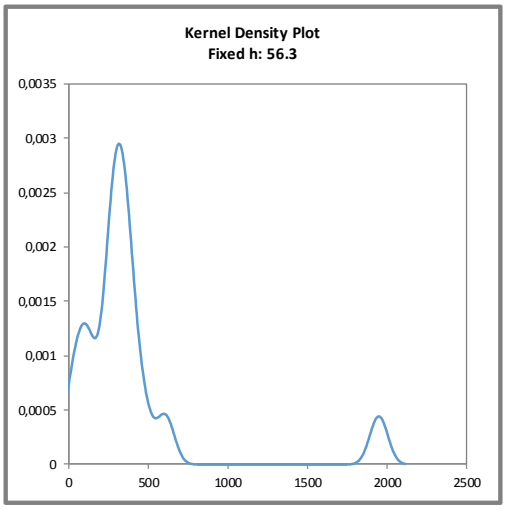


Abb. / Fig. 12:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with two shoulders at <200 mg/kg and approx. 600 mg/kg as well as a side peak at about 2000 mg/kg, which is due to a single result above the target range.

Characteristics: Quantitative evaluation ELISA Casein

Spiking Level Sample

Statistic Data	**All Results [mg/kg]**	**Method AQ [mg/kg]**
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results°	14	7
Number of outliers	2	-
Mean	306	567
Median	307	356
Robust Mean (X_{pt})	**300**	**379**
Robust standard deviation (S*)	**140**	**170**
Target range:		
Target standard deviation σ_{pt}	**75,1**	**94,8**
lower limit of target range	**150**	**190**
upper limit of target range	**451**	**569**
Quotient S/σ_{pt}*	*1,9*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*46,8*	*80,5*
Results in the target range	*9*	*5*
Percent in the target range	*64*	*71*

° without results No. 1 and 12 for XptALL (as outlier excluded)

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

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

The evaluation of all methods as well as of method AQ showed a normal variability of results, with quotients S/σpt below 2,0.*
The robust standard deviations are in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 115% and 145% of the spiking level of casein to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Casein" p.40).

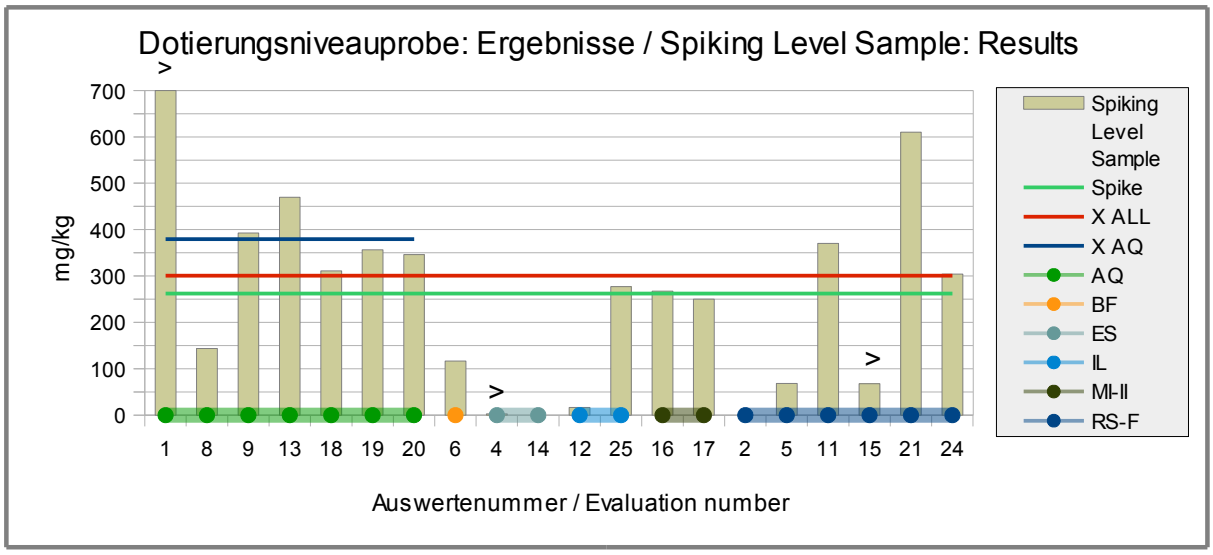


Abb./Fig. 13: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

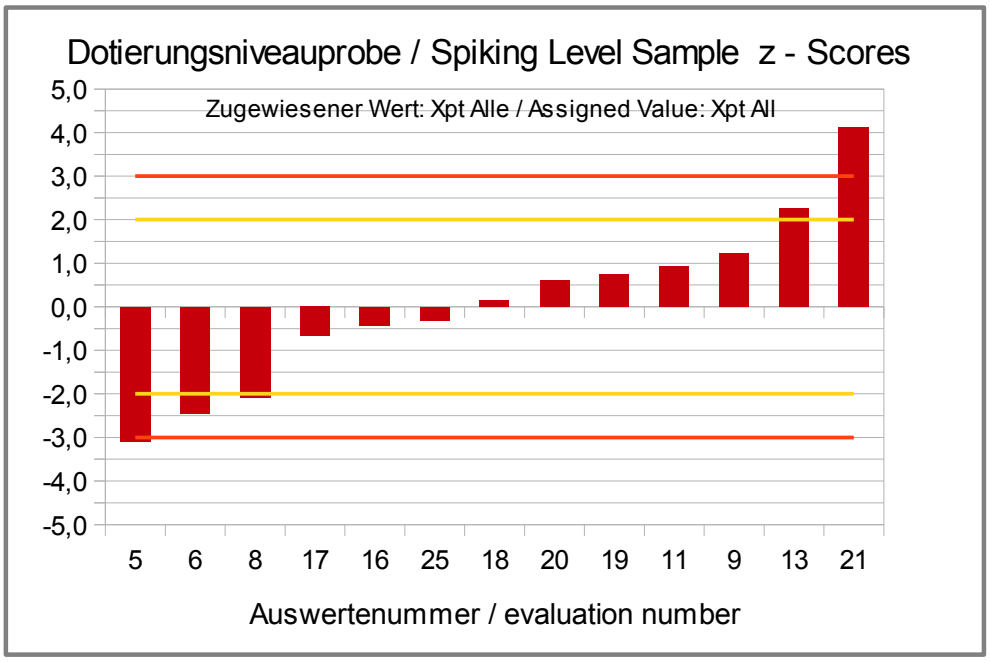


Abb./Fig. 14:
z-Scores (ELISA Results Casein)
Assigned value robust mean of all results

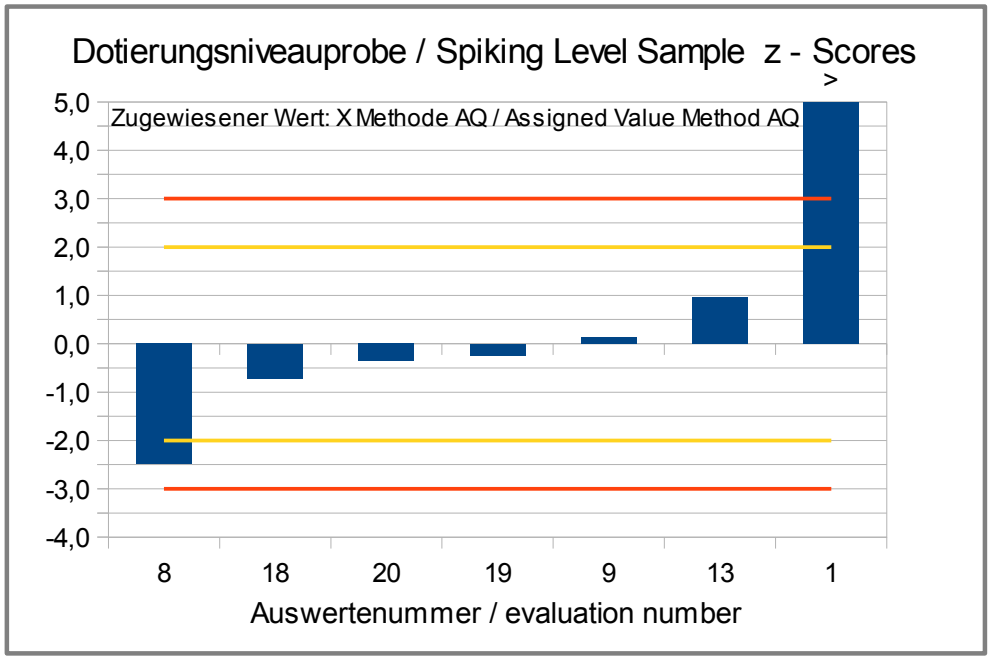


Abb./Fig. 15:
z-Scores (ELISA Results Casein)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Recovery Rates ELISA for Casein: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
1	1950	744	1475	531	AQ	
8	144	**55**	332	**119**	AQ	
9	393	**150**	424	153	AQ	
13	470	179	410	**147**	AQ	
18	311	**119**	498	179	AQ	
19	356	**136**	425	153	AQ	
20	346	**132**	436	157	AQ	
6	117	45	198	**71**	BF	
4	>2,56		>2,56		ES	Result converted °
14			47,7	17	ES	
12	16,5	6,3	163	**59**	IL	
25	277	**106**	477	172	IL	
16	268	**102**	302	**109**	MI-II	Result converted °
17	250	**95**	340	**122**	MI-II	
2			7,90	2,8	RS-F	
5	68,3	26	77,4	28	RS-F	
11	370	**141**	340	**122**	RS-F	
15	>67,5		>67,5		RS-F	
21	610	233	640	230	RS-F	
24	304	**116**	200	**72**	RS-F	

° calculation see p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**10**	Number in RA	**8**
Percent in RA	**63**	Percent in RA	**44**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For the spiking level sample 63% (10) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 44% (8) of the recovery rates were within the range of acceptance.

4.2.2 ELISA Results: Milk (as milk protein)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
6	negative	0	positive	316	2/2 (100%)	BF	Result converted °, method casein-specific
25	negative	0	positive	263	2/2 (100%)	IL	
22	negative		positive	33,0	2/2 (100%)	RS-F	Result converted °
24	negative	<2,5	positive	261	2/2 (100%)	RS-F	
4	negative	<0,825	positive	>8,25	2/2 (100%)	VT	Result converted °
7	negative		positive	>25,0	2/2 (100%)	VT	
14	negative	<LOD	positive	518	2/2 (100%)	VT	Result converted °

° calculation see p. 20

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

Methods:
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample B

An evaluation of the quantitative results was not carried out because there were only a few, highly inhomogeneous results.

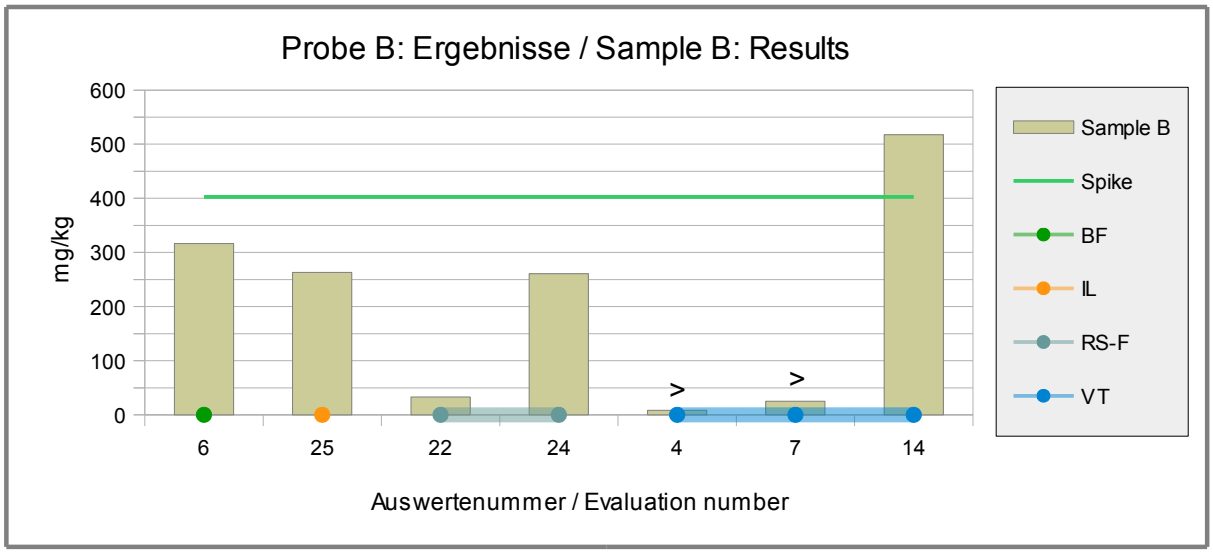


Abb./Fig. 16: ELISA Results Milk (as milk protein)
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation of results: Spiking level sample

An evaluation of the quantitative results was not carried out because there were to few results.

Evaluation number	Milk protein	Milk protein	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
6	positive	189		BF	Result converted °, method casein-specific
25	positive	191		IL	
22	positive	29,7		RS-F	Result converted °
24	positive	350		RS-F	
4	positive	>8,25		VT	Result converted °
7				VT	
14				VT	

° calculation see p. 20

Number positive	5
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methoden:

BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking level sample only positive results were obtained.

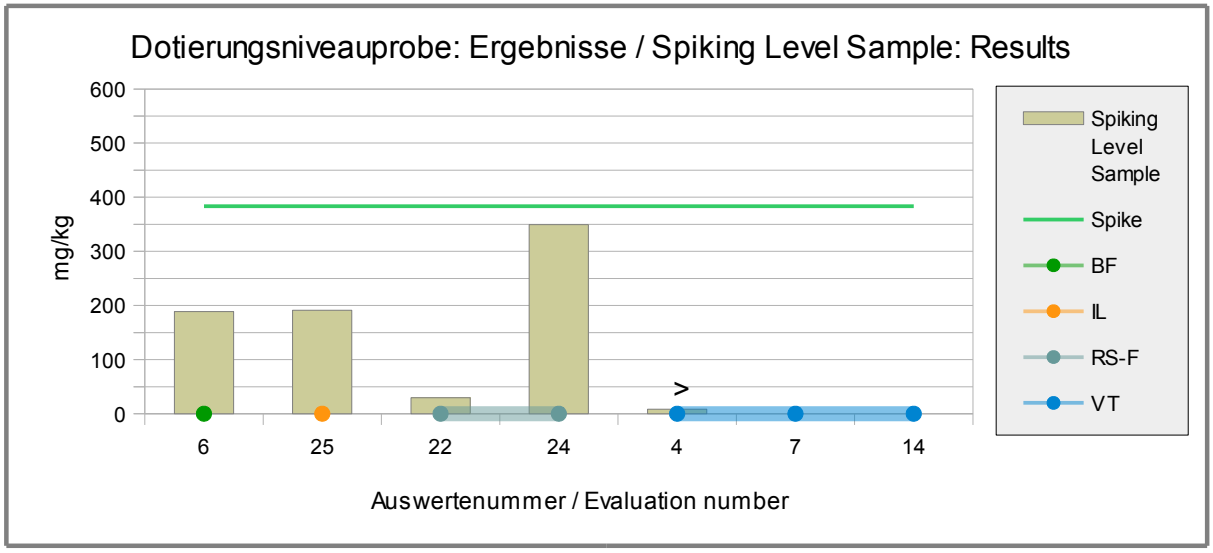


Abb./Fig. 17: ELISA Results Milk (as milk protein)
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	189	49	316	**79**	BF	Result converted °, method casein-specific
25	191	**50**	263	**65**	IL	
22	29,7	7,8	33,0	8,2	RS-F	Result converted °
24	350	**91**	261	**65**	RS-F	
4	>8,25		>8,25		VT	Result converted °
7			>25,0		VT	
14			518	**129**	VT	Result converted °

° calculation see p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**2**	Number in RA	**4**
Percent in RA	**50**	Percent in RA	**80**

Methods:
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:

For the spiking level sample 2 of the 4 participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 80% (4) of the recovery rates were within the range of acceptance.

4.3 Proficiency Test Wheat (Gluten / Wheat)

4.3.1 ELISA Results: Gluten

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		
23	negative	<LOD	positive	83,7	2/2 (100%)	AQ	
1	positive	9	positive	68,0	1/2 (50%)	AQ-G12	
18a	positive	<4	positive	44,0	1/2 (50%)	AQ-G12	
20	positive	<LOQ	positive	31,0	1/2 (50%)	AQ-G12	
6	negative	<ROQ	positive	45,5	2/2 (100%)	BF	
17a	negative	<3,12	positive	66,0	2/2 (100%)	EF-R5	
12	negative	<4,0	positive	124	2/2 (100%)	IL	
25	negative	0	positive	55,0	2/2 (100%)	IL	
2	negative		positive	32,3	2/2 (100%)	RS	
3	negative	< 5,0	positive	54,8	2/2 (100%)	RS	
4a	negative	<5	positive	48,0	2/2 (100%)	RS	
7	negative		positive	38,5	2/2 (100%)	RS	
8	negative	<5	positive	48,5	2/2 (100%)	RS	
9	negative		positive	55,4	2/2 (100%)	RS	
11	negative		positive	44,0	2/2 (100%)	RS	
13	negative	<5,0	positive	66,0	2/2 (100%)	RS	
15	negative		positive	46,5	2/2 (100%)	RS	
16	negative		positive	31,7	2/2 (100%)	RS	
17b	negative	<5	positive	47,0	2/2 (100%)	RS	
18b	positive	<5	positive	57,0	1/2 (50%)	RS	
19	negative	< 5	positive	37,6	2/2 (100%)	RS	
21	negative	<10	positive	39,0	2/2 (100%)	RS	
22	negative		positive	60,0	2/2 (100%)	RS	
24	negative	<5	positive	57,6	2/2 (100%)	RS	
5	negative	1,31	positive	35,5	2/2 (100%)	RS-F	Sample A: positive result >LOD
4b	negative	<2,5	positive	>20	2/2 (100%)	RS-FS	
14	negative	<LOD	positive	44,9	2/2 (100%)	VT-R5	

	Sample A		Sample B	
Number positive	4		27	
Number negative	23		0	
Percent positive	15		100	
Percent negative	85		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
AQ-G12 = AgraQuant G12, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-FS= Ridascreen® Fast sensitive, R-Biopharm
VT-R5 = Veratox, Neogen

Comments:
The consensus values are in qualitative agreement with the spiking of sample B. For sample A four positive results were obtained in the range of below 10 mg/kg.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
23	83,7	2,7		AQ	
1	68,0	1,5		AQ-G12	
18a	44,0	-0,47		AQ-G12	
20	31,0	-1,5		AQ-G12	
6	45,5	-0,35		BF	
17a	66,0	1,3		EF-R5	
12	124	6,0		IL	
25	55,0	0,42		IL	
2	32,3	-1,4	-1,3	RS	
3	54,8	0,40	0,60	RS	
4a	48,0	-0,15	0,03	RS	
7	38,5	-0,91	-0,77	RS	
8	48,5	-0,11	0,07	RS	
9	55,4	0,45	0,65	RS	
11	44,0	-0,47	-0,31	RS	
13	66,0	1,3	1,5	RS	
15	46,5	-0,27	-0,10	RS	
16	31,7	-1,5	-1,3	RS	
17b	47,0	-0,23	-0,06	RS	
18b	57,0	0,58	0,78	RS	
19	37,6	-1,0	-0,8	RS	
21	39,0	-0,87	-0,73	RS	
22	60,0	0,82	1,0	RS	
24	57,6	0,63	0,84	RS	
5	35,5	-1,1		RS-F	
4b	>20			RS-FS	
14	44,9	-0,39		VT-R5	

Methods:

AQ = AgraQuant, RomerLabs
AQ-G12 = AgraQuant G12, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-FS= Ridascreen® Fast sensitive, R-Biopharm
VT-R5 = Veratox, Neogen

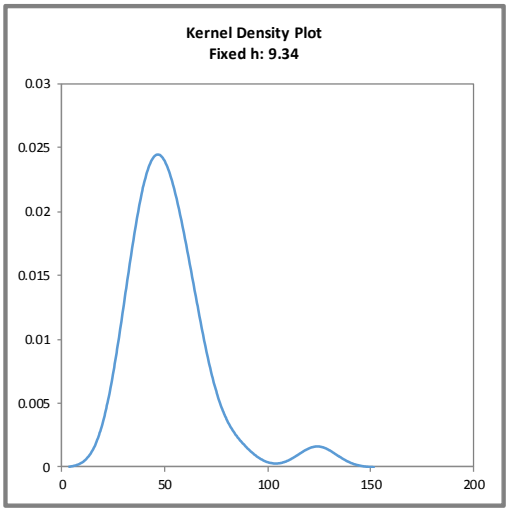


Abb. / Fig. 18:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a side peak at approx. 125 mg/kg (method IL) due to a single result above the target range.

Characteristics: Quantitative evaluation ELISA Gluten

Sample B

Statistic Data	**All Results [mg/kg]**	**Method RS** [mg/kg]
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	26	16
Number of outliers	-	-
Mean	52,4	47,7
Median	47,5	47,5
Robust Mean (X_{pt})	**49,8**	**47,7**
Robust standard deviation (S*)	**13,9**	**11,4**
Target range:		
Target standard deviation σ_{pt}	**12,5**	**11,9**
lower limit of target range	**24,9**	**23,8**
upper limit of target range	**74,7**	**71,5**
Quotient S/σ_{pt}*	*1,1*	*0,95*
Standard uncertainty $U_{(Xpt)}$	*3,40*	*3,55*
Results in the target range	*24*	*16*
Percent in the target range	*92*	*100*

Method:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

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

The evaluation of all methods as well as method RS showed a normal to low variability of results, with a quotient S/σpt below 2,0 each.*
The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 156% and 149% of the spiking level of gluten to sample B and thus at the upper limit of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Gluten" p.55).

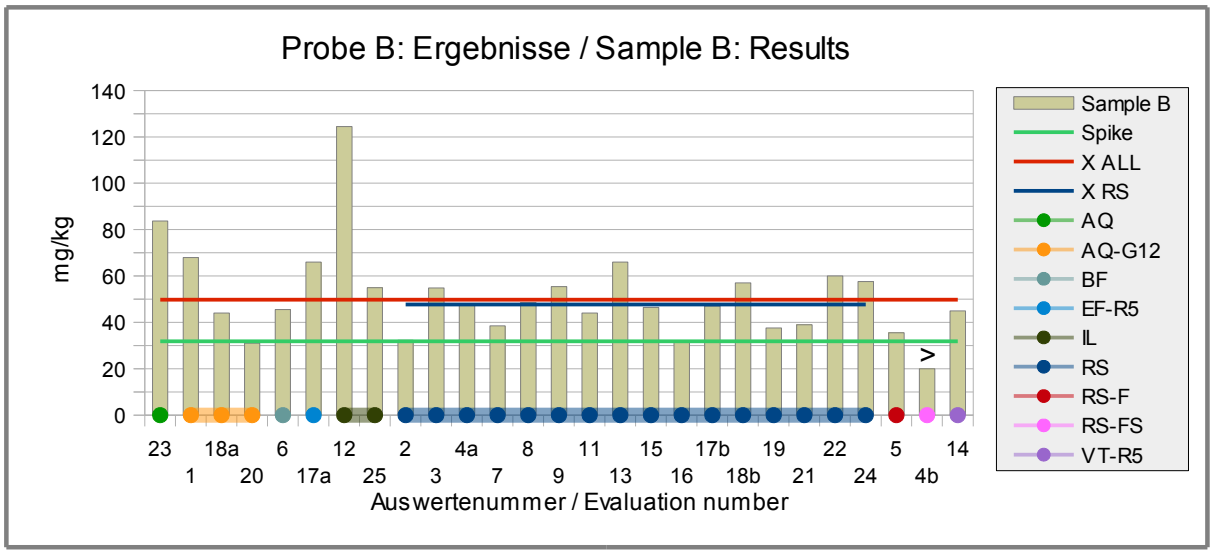


Abb./Fig. 19: ELISA Results Gluten
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS
round symbols = Applied methods (see legend)

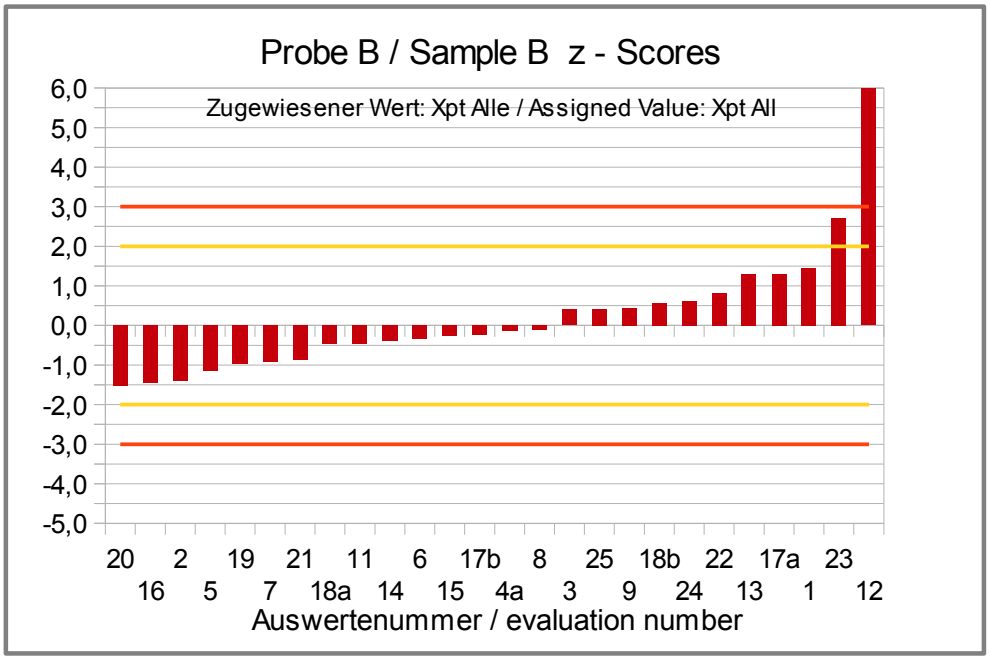


Abb./Fig. 20:
z-Scores (ELISA Results Gluten)
Assigned value robust mean of all results

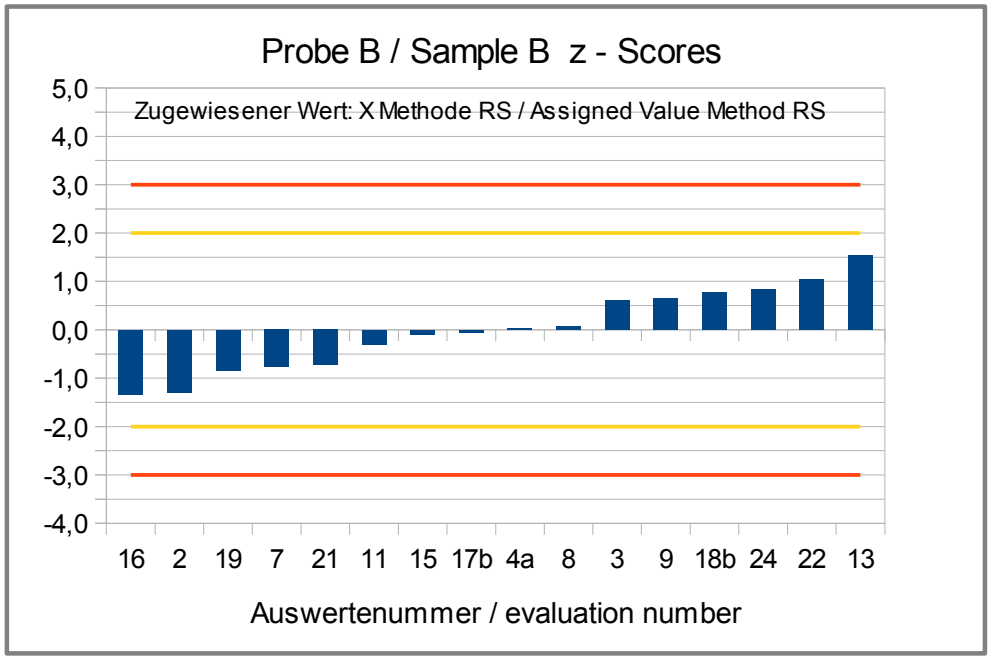


Abb./Fig. 21:
z-Scores (ELISA Results Gluten)
Assigned value robust mean of method RS(R-Biopharm, Ridascreen)

Quantitative Valuation of results: Spiking level sample

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
23	127	5,4		AQ	
1	39,1	-1,1		AQ-G12	
18a	37,0	-1,3		AQ-G12	
20	29,0	-1,9		AQ-G12	
6	53,4	-0,05		BF	
17a	66,0	0,88		EF-R5	
12	209	11,5		IL	
25	88,0	2,5		IL	
2				RS	
3	63,5	0,69	0,84	RS	
4a	49,0	-0,38	-0,26	RS	
7	38,0	-1,2	-1,1	RS	
8	49,3	-0,36	-0,24	RS	
9	51,6	-0,19	-0,06	RS	
11	44,0	-0,75	-0,64	RS	
13	74,0	1,5	1,6	RS	
15	61,2	0,52	0,67	RS	
16	33,9	-1,5	-1,4	RS	
17b	48,0	-0,45	-0,34	RS	
18b	71,0	1,2	1,4	RS	
19	48,4	-0,42	-0,31	RS	
21	40,0	-1,0	-0,95	RS	
22	50,0	-0,30	-0,19	RS	
24	66,0	0,88	1,0	RS	
5	44,6	-0,70		RS-F	
4b	>20			RS-FS	
14				VT-R5	

Methods:

AQ = AgraQuant, RomerLabs
AQ-G12 = AgraQuant G12, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-FS= Ridascreen® Fast sensitive, R-Biopharm
VT-R5 = Veratox, Neogen

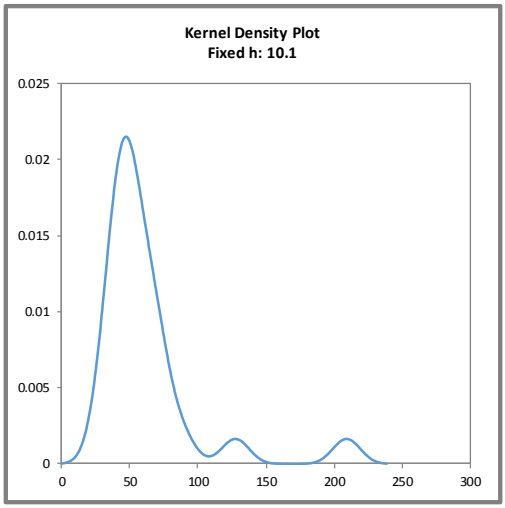


Abb. / Fig. 22:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with two side peaks at approx. 130 mg/kg and 210 mg/kg due to single results out of the target range (method AQ and IL).

Characteristics: Quantitative evaluation ELISA Gluten

Spiking Level Sample

Statistic Data	**All Results [mg/kg]**	**Method RS** [mg/kg]
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	24	15
Number of outliers	-	-
Mean	61,7	52,5
Median	49,7	49,3
Robust Mean (X_{pt})	**54,1**	**52,4**
Robust standard deviation (S*)	**17,6**	**13,5**
Target range:		
Target standard deviation σ_{pt}	**13,5**	**13,1**
lower limit of target range	**27,1**	**26,2**
upper limit of target range	**81,2**	**78,7**
Quotient S/σ_{pt}*	*1,3*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*4,49*	*4,35*
Results in the target range	*21*	*15*
Percent in the target range	*88*	*100*

Method:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

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

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

The robust means of the evaluations were 149% and 145% of the spiking level of gluten to the spiking level sample and thus within the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Gluten" p.55).

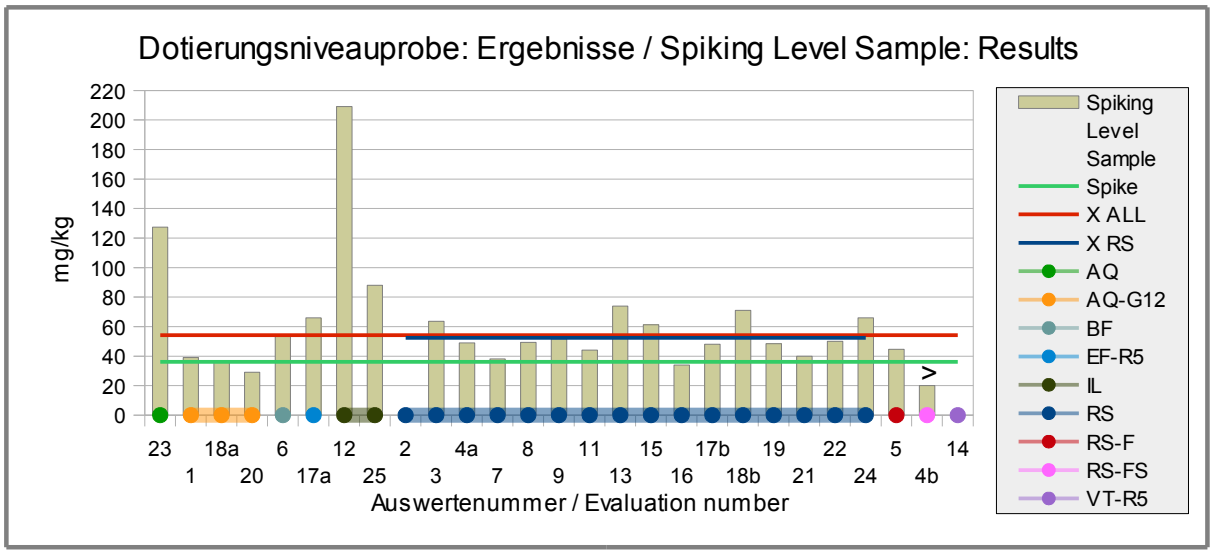


Abb./Fig. 23: ELISA Results Gluten
green line = Spiking level
blue line = Assigned value robust mean results method RS
red line = Assigned value robust mean of all results
round symbols = Applied methods (see legend)

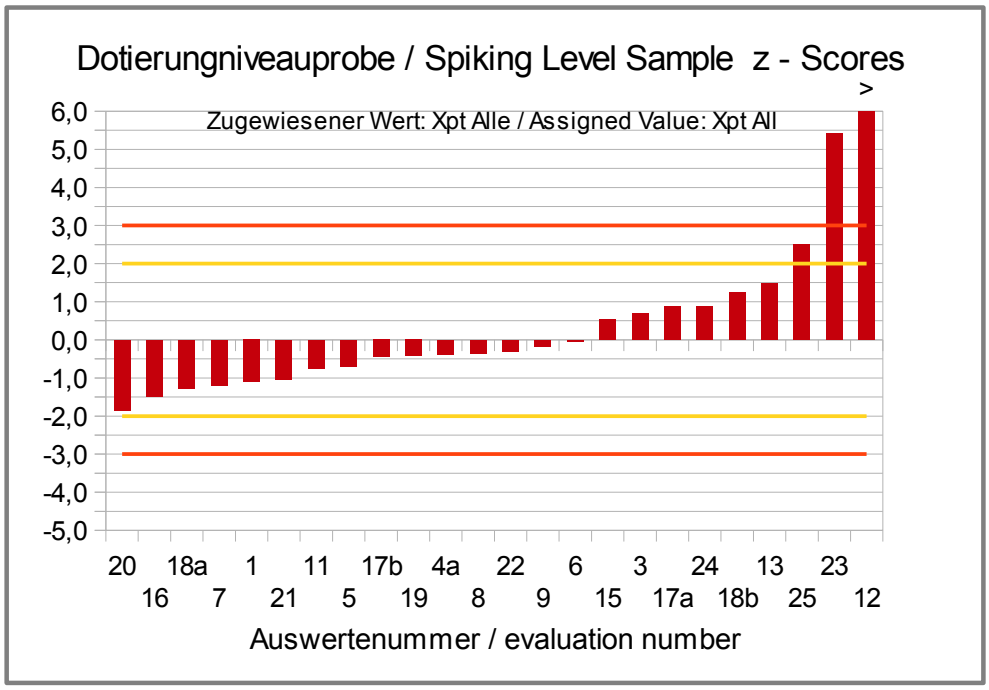
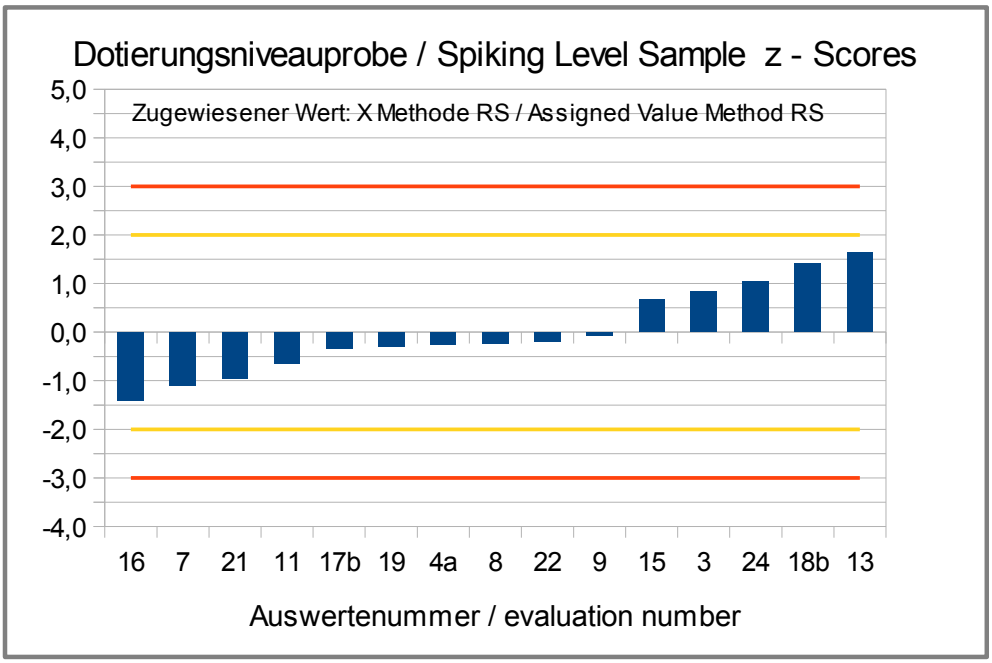


Abb./Fig. 24:
z'-Scores (ELISA Results Gluten)
Assigned value robust mean of all results



z-Scores (ELISA Results Gluten)
Assigned value robust mean of method RS (R-Biopharm, Ridascreen)

Recovery Rates ELISA for Gluten: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
23	127	352	83,7	262	AQ	
1	39,1	**108**	68,0	213	AQ-G12	
18a	37,0	**102**	44,0	**138**	AQ-G12	
20	29,0	**80**	31,0	**97**	AQ-G12	
6	53,4	**148**	45,5	**143**	BF	
17a	66,0	182	66,0	207	EF-R5	
12	209	578	124	390	IL	
25	88,0	243	55,0	172	IL	
2			32,3	**101**	RS	
3	63,5	175	54,8	172	RS	
4a	49,0	**135**	48,0	**150**	RS	
7	38,0	**105**	38,5	**121**	RS	
8	49,3	**136**	48,5	152	RS	
9	51,6	**143**	55,4	174	RS	
11	44,0	**122**	44,0	**138**	RS	
13	74,0	204	66,0	207	RS	
15	61,2	169	46,5	**146**	RS	
16	33,9	**94**	31,7	**99**	RS	
17b	48,0	**133**	47,0	**147**	RS	
18b	71,0	196	57,0	179	RS	
19	48,4	**134**	37,6	**118**	RS	
21	40,0	**110**	39,0	**122**	RS	
22	50,0	**138**	60,0	188	RS	
24	66,0	182	57,6	181	RS	
5	44,6	**123**	35,5	**111**	RS-F	
4b	>20		>20		RS-FS	
14			44,9	**141**	VT-R5	

RA**	50-150 %	RA**	50-150 %
Number in RA	**15**	Number in RA	**14**
Percent in RA	**63**	Percent in RA	**54**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
AQ-G12 = AgraQuant G12, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-FS= Ridascreen® Fast sensitive, R-Biopharm
VT-R5 = Veratox, Neogen

Comments:

63% (15) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150% with the spiking level sample. For the spiked food matrix sample B 54% (14) of the recovery rates were within the range of acceptance.

4.3.2 PCR Results: Wheat

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		
15	negative		positive		2/2 (100%)	SFA-ID	

	Sample A		Sample B	
Spiking	negative		positive	

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

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

Qualitative valuation of results: Spiking level sample

Evaluation number	Gluten	Gluten	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
15	positive			SFA-ID	

Spiking Level Sample	
Spiking	positive

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

Comments:
The result of the participant is in qualitative agreement with the spiking of the spiking level sample.

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: β-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	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	1	24.04.19	-	<LOD	-	18,65	-	18,76	0,0015	0,01		beta-Lactoglobulin	AgraQuant ELISA β-Lactoglobulin COLAL1048, RomerLabs
AQ	18	08.04.19	negative	<LOD	positive	18	positive	16	0,0015	0,01	50	beta-Lactoglobulin	AgraQuant ELISA β-Lactoglobulin COLAL1048, RomerLabs
AQ	20	05.04.19	negative	< LOD	positive	16	positive	16,1	0,0015	0,01	50	beta-Lactoglobulin	AgraQuant ELISA β-Lactoglobulin COLAL1048, RomerLabs
ES	4		negative	<0,1	positive	>1	positive	>1		0,1		beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
ES	13	11.04.19	negative	<0.10	positive	98	positive	93		0.10		beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
ES	14	09.04.19	Negative	<LOD	Pos	136,8	-	Not tested	0,05	0,1		Beta-lactoglobulin	ELISA Systems - Beta-lactoglobulin
IL	12		negative	<0,01	positive	207,3	positive	14,5	0,0015	0,01		beta-Lactoglobulin	Immunolab Beta-Lactoglobulin ELISA
IL	25	21.03.19	negative	0	positive	14,2	positive	12,9				beta-Lactoglobulin	Immunolab Beta-Lactoglobulin ELISA
MI-II	16	20.03.19	negative		positive	325,4	positive	310		0,31		Milk proteins, total	Morinaga Beta-lactoglobulin ELISA Kit II (M2112)
MI-II	17	22.03.	negative	<0,031	positive	32	positive	31	0,031	0,031		beta-Lactoglobulin	Morinaga Beta-lactoglobulin ELISA Kit II (M2112)
RS	10	11.04.19	negative	<2.63	positive	36,4	positive	28,9	0,79	2,63	31	beta-Lactoglobulin	Ridascreen® β-Lactoglobulin R4901, R-Biopharm
RS-F	2		negative		positive	10	-					beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	5	24/04	negative	0,07339	positive	4,56484	positive	4,53287	0,04	0,167		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	8	18.04.19	negative	<1.5	positive	36	positive	36,7				beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	9	17.04.19	negative		positive	29,1	positive	33,8	0,5	0,5		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	11	22.03.19	negative		positive		positive		0,2	0,5			Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	15		negative		positive	> 4,5	positive	> 4,5		0,17			Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	21	04.02.19	negative	<0,2	positive	>4,5	positive	>4,5	0,2	0,2	-	beta-Lactoglobulin	other: please fill in!
RS-F	24	05.04.19	negative	<0,167	positive	18,87	positive	29,71		<0,167		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm

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

***Continuation ELISA β-Lactoglobulin*:**

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	1	polyclonal	1.0g sample + 20ml diluted extraction buffer. Shake 15 min in 60°C w aterbath. Centrifuge 10min.	yes	
AQ	18	Beta-Lactoglobulin		yes	
AQ	20	Beta-Lactoglobulin	aqueous buffer 60 ° C / 15 minutes orbital shaker	no	
ES	4			no	
ES	13			yes	
ES	14		Extraction: Room temperature PBS buffer (pH check)/shaking w ater bath at 60C for 15 min/ Determination: 4 parameter curve	yes	
IL	12				
IL	25				
MI-II	16				
MI-II	17	recognizes cow 's milk β-lactoglobulin	according to manufacturer's instructions	yes	
RS	10	Anti-BLG	w ashing buffer, 10 minutes, 50°C	No	
RS-F	2			yes	
RS-F	5		Extraction solution: Extractor 2+Allergen extraction buffer containing Additive 1, time: approx. 30 min., temperature: 100 °C		
RS-F	8			yes	
RS-F	9	beta-Lactoglobulin		yes	Remark: Article no. is now R4912
RS-F	11	specific antibodies to β-lactoglobulin	Weigh in 1 g sample and add 4 ml prepared Extractor 2, mix vigorously, close the vial and cook it for 10 min at 100 °C in a w ater bath, let the sample cool dow n shortly, pre-heat the A-AEP to 60 °C , add 16 ml heated (60 °C) A-AEP to the cooked sample. Mix vigorously (extract for 10 min at 60 °C in a w ater bath), cool dow n, centrifuge for 10 min / at high speed in a microcentrifuge. Dilute the particle free supernatant 1:5 (1+4) w ith diluted Allergen Extraction buffer, w ithout Additive 1	no	
RS-F	15			yes	
RS-F	21	β-Lactoglobulin of cow 's milk	Extractor 2+A-AEP/90 min/20-25°C	yes	Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	24			no	the sample that agglutinates and forms paste. it w eighs one tenth of the usual. there can be a lot of uncertainty

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	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	1	25.04.19	-	<LOD	-	1475	-	1950	0,04	0,2		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	8	15.04.19	negative	<1.5	positive	332	positive	144				Casein	AgraQuant Plus Casein COKAL1248F, RomerLabs
AQ	9	20.03.19	negative		positive	424	positive	393	0,2	0,2		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	13	11.04.19	negative	<0.20	positive	410	positive	470		0.20		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	18	27.03.19	negative	<LOD	positive	498	positive	311	0,04	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	19		negative	< 0,2	positive	424,64	positive	356,29	0,04	0,2		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	20	05.04.19	negative	< LOD	positive	436	positive	346	0,04	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
BF	6	25/04	negative	0	positive	197,6	positive	116,7	0,12	0,5		Casein	MonoTrace Milk (Casein) ELISA kit, BioFront Technologies
ES	4		negative	<1	positive	>10	positive	>10		1		Skimmed milk powder	ELISA Systems Casein ESCASPRD-48
ES	14	09.04.19	Negative	<LOD	Pos	47,7	-	Not tested	0,14	0,28		Total casein	ELISA Systems - Casein
IL	12		negative	<0,2	positive	163,2	positive	16,5	0,04	0,2		Casein	Immunolab Casein ELISA
IL	25	21.03.19	negative	0	positive	477	positive	277				Casein	Immunolab Casein ELISA
MI-II	16	20.03.19	negative		positive	378,1	positive	334,5		0,31		Milk proteins, total	Morinaga Casein ELISA Kit II (M2113)
MI-II	17	22.03.	negative	<0,25	positive	340	positive	250	0,25	0,25		Casein	Morinaga Casein ELISA Kit II (M2113)
RS-F	2		negative		positive	7,9	-					Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	5	24/04	negative	0,49419	positive	77,4409	positive	68,2876	0,71	2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	11	23.04.19	negative		positive	340	positive	370	1	2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	15		negative		positive	>67,5	positive	>67,5		2,5			Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	21	04.02.19	negative	<3,0	positive	640	positive	610	3	3	-	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	24	04.04.19	negative	<2,5	positive	199,87	positive	303,99		<2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Casein:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	1	polyclonal	0.5g sample +10ml prediluted and heated extraction buffer. Shake 15 min. Centrifuge 10 min.	YES	
AQ	8			yes	
AQ	9	Casein		yes	
AQ	13			YES	
AQ	18	Casein		yes	
AQ	19	Casein	Extraction solution, 15 min at 60 ° C, 1: 500 dilution	no	
AQ	20	Casein	aqueous buffer / 15 minutes / 60 ° C	no	
BF	6	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	no	
ES	4			no	
ES	14		Extraction: Room temperature PBS buffer (ph check)/shaking w ater bath at 60C for 15 min/ Determination: 4 parameter curve	yes	
IL	12				
IL	25				
MI-II	16				
MI-II	17	recognizes cow 's milk casein	according to manufacturer's instructions	yes	
RS-F	2			yes	
RS-F	5		Extraction solution: Extractor 2+Allergen extraction buffer containing Additive 1, time: approx. 30 min., temperature: 100 °C		
RS-F	11	specific antibodies to casein	Take 1 g of sample and add 4 ml prepared Extractor 2, mix vigorously, close the vial and cook it for 10 min at 100 °C in a w ater bath. Let the sample cool dow n shortly, add 16 ml heated (60 °C) A-AEP to the cooked sample. Mix vigorously (shaker) and extract for 10 min at 60 °C in a w ater bath. Cool dow n, centrifuge for 10 min / 2500 g and/or filter.Dilute the particle free supernatant or the filtrate 1:5 (1+4) w ith diluted Allergen Extraction buffer, w ithout Additive 1	yes	
RS-F	15			yes	
RS-F	21	casein of cow 's milk	Extractor 2+A-AEP/90 min/20-25°C	yes	
RS-F	24			no	the sample that agglutinates and forms paste. it w eighs one tenth of the usual. there can be a lot of uncertainty

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	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
BF	6	25/04	negative	0	positive	958,24	positive	571,83	0,12	0,5		Skimmed milk powder	MonoTrace Milk (Casein) ELISA kit, BioFront Technologies
IL	25	21.03.19	negative	0	positive	263	positive	191				milk proteins, total	Immunolab Milk ELISA
RS-F	22		negative		positive	100	positive	90	1	10	50	food	Ridascreen Fast Milk
RS-F	24	08.04.19	negative	<2,5	positive	260,56	positive	349,57		<2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
VT	4		negative	<2,5	positive	>25	positive	>25		2,5		Skimmed milk powder	Veratox Total Milk Allergen, Neogen
VT	7		negative		positive	>25,0	-		2,5			Milk proteins, total	Veratox Total Milk Allergen, Neogen
VT	14	04.04.19	Negative	<LOD	Pos	1568,7	-	Not tested	1	2,5		Non-fat dried milk	Neogen Veratox for Total milk

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BF	6	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	no	Results were converted to milk from casein
IL	25				
RS-F	22			yes	
RS-F	24			no	the sample that agglutinates and forms paste. it weighs one tenth of the usual. there can be a lot of uncertainty
VT	4			yes	
VT	7		extraction solution/15 min./60 °C	yes	
VT	14		Extraction: 60C pre-heated PBS buffer/shaking water bath at 60C for 15 min/ Determination: 4 parameter curve	yes	

5.1.4 ELISA: Gluten

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	23	16.03.19	-	<LOD	-	83,67	-	127,3	0,6	5	11	Gluten	AgraQuant ELISA Gluten COKAL0248, RomerLabs
AQ	1	24.04.19	-	9	-	68	-	39,1	2	4		Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
AQ	20	09.04.19	positive	< LOQ	positive	31	positive	29	2	4	40	Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
AQ	18a	08.04.19	positive	<4	positive	44	positive	37	4	2	40	Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
BF	6	25/04	negative	bROQ	positive	45,5	positive	53,4	0,36	2			MonoTrace Gluten ELISA kit, BioFront Technologies
EF-R5	17a	28.3.	negative	<3,12	positive	66	positive	66	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins
IL	12		negative	<4,0	positive	124,4	positive	209,1	0,3	2		Gluten	Immunolab Gliadin/Gluten ELISA
IL	25	08.04.19	negative	0	positive	55	positive	88				Gluten	Immunolab Gliadin/Gluten ELISA
RS	2		negative		positive	32,3	-					Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	3	15.04.19	negative	< 5,0	positive	54,8	positive	63,5				Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	4a		negative	<5	positive	48	positive	49		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	7		negative		positive	38,5	positive	38	5			Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8	08.05.19	negative	<5	positive	48,5	positive	49,3				Gluten	Ridascreen➢Gliadin r-biopharm R7001
RS	9	10.04.19	negative		positive	55,4	positive	51,6	5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	11	27.03.19	negative		positive	44	positive	44	2	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	13	11.04.19	negative	<5.0	positive	66	positive	74		5.0		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	15		negative		positive	46,5	positive	61,2		5			Ridascreen® Gliadin R7001, R-Biopharm
RS	16	21.03.19	negative		positive	31,7	positive	33,9		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	17b	22.03.	negative	<5	positive	47	positive	48	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	18b	03.04.19	positive	<5	positive	57	positive	71	5	1	50	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	19		negative	< 5	positive	37,62	positive	48,42	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	21	04.02.19	negative	<10	positive	39	positive	40	10	10	28	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	22		negative		positive	60	positive	50	2	20	50	Food	Ridascreen Gluten
RS	24	29.03.19	negative	<5	positive	57,62	positive	66,02		<5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS-F	5	24/04	negative	1,30976	positive	35,5324	positive	44,6136	1	10		Gluten	Ridascreen® FAST Gliadin R7002, R-Biopharm
RS-FS	4b	25.04.2019	negative	<2,5	positive	>20	positive	>20		2,5		Gluten	Ridascreen® Gliadin R7051, R-Biopharm
VT-R5	14	02.04.19	Negative	<LOD	Pos	44,9	-	Not tested	4,3	5		Gluten	Neogen Veratox for Gliadin R5

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Gluten:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	23				
AQ	1	monoclonal	0.25g sample + 2.5ml extraction solution. Incubate 50C for 40min. Cool and add 7.5ml 80% ethanol. Shake 60min at room temperature. Centrifuge 10 min.	YES	
AQ	18a	Gluten		yes	
AQ	20	Gluten	Extraction buffer / 40 minutes 50 ° C / ethanol / 60 minutes orbital shaker	no	
BF	6	Monoclonal antibody-based assay	1:40 extraction ratio in MonoTrace Gluten Extraction Buffer/1 hour/60C	no	
EF-R5	17a	Mendez R5	according to manufacturer's instructions	yes	
IL	12				
IL	25				
RS	2			yes	
RS	3	Monoclonal R5	80% ethanol / 1h / room temperature	yes	LAB_AR results
RS	4a	R5		yes	
RS	7		monoclonal antibody R5	yes	
RS	8			yes	
RS	9	Gliadine (R5-Antikörper)		yes	
RS	11	specific R5 antibodies against gliadins	Weigh 0.25 g of the homogenized sample and add 2.5 ml of the Cocktail, close the vial and mix w ell. Incubate for 40 min at 50 °C, let the sample cool dow n and then mix it w ith 7.5 ml 80 % ethanol. Close the vial and shake for 1 h up side dow n or by a rotator at room temperature. Centrifuge: 10 min, at least 2500 g, at room temperature and 2 ml of the extract can be centrifuged w ith high speed for 10 min in reaction caps by using a microcentrifuge. Transfer the supernatant in a screw top vial, dilute the sample 1:12.5 w ith diluted sample diluent: the final dilution factor is 500	yes	
RS	13			YES	
RS	15			yes	
RS	16				
RS	17b	Mendez R5	according to manufacturer's instructions	yes	
RS	18b	Gliadin		yes	
RS	19	Gliadin	Extraction w ith Cocktail solution, and Ethanol, 40 min at 50°C, 1:500 dilution	no	
RS	21	R5	Cocktail+etanol 80%/150min/20-25°C	yes	
RS	22			yes	
RS	24			no	
RS-F	5	R5	Extraction solution: Coctail (patented), time: approx. 2 hours, temperature: 50 °C		
RS-FS	4b	R5		no	
VT-R5	14		Extraction: Incubation at 50C w ith cocktail solution / Addition of ethanol and shaking for 1 hour at room temperature/ Sample is diluted w ith PBS before plating/ Determination: 4 parameter curve	yes	

5.1.5 PCR: Wheat

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
SFA-ID	15		-		positive		positive		0,4				Sure Food Allergen ID, R-Biopharm / Congen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
SFA-ID	15			no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 03-2019 Sample B

Weight whole sample	2,82	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	15,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	53	21,2
2	5,07	59	23,3
3	5,00	58	23,2
4	5,03	55	21,9
5	5,06	55	21,7
6	5,09	57	22,4
7	5,06	52	20,6
8	5,02	49	19,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,7	Particles
Standard deviation	3,24	Particles
χ^2 (CHI-Quadrat)	1,34	
Probability	**99**	%
Recovery rate	140	%

Normal distribution		
Number of samples	8	
Mean	21,7	mg/kg
Standard deviation	1,28	mg/kg
rel. Standard deviaton	5,91	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,59**	
Recovery rate	140	%

Microtracer Homogeneity Test
DLA 03-2019 Spiking Level Sample

Weight whole sample	1,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	82	32,8
2	5,06	84	33,2
3	4,99	83	33,3
4	5,09	96	37,7
5	5,02	85	33,9
6	5,03	82	32,6
7	5,02	81	32,3
8	5,01	77	30,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	83,7	Particles
Standard deviation	5,05	Particles
χ^2 (CHI-Quadrat)	2,13	
Probability	**95**	%
Recovery rate	118	%

Normal distribution		
Number of samples	8	
Mean	33,3	mg/kg
Standard deviation	2,01	mg/kg
rel. Standard deviaton	6,03	%
Horwitz standard deviation	9,44	%
HorRat-value	**0,64**	
Recovery rate	118	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 03-2019***
PT name	***Allergens III: β-Lactoglobulin, Casein and Gluten in Infant Food***
Sample matrix (processing)	***Samples A + B:*** *Cereal pap powder, "gluten-free"/ ingredients: Rice flour 70%, maize flour 20%, sorghum whole meal 10%, thiamine and other food additives and allergenic foods skimmed milk powder, whey powder and wheat flour (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each + 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C) Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: β-Lactoglobulin, Casein and Gluten (Gluten-containing Cereals) Samples A + B: < 1000 mg/kg Spiking Level Sample: < 1000 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest April 26th 2019.***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*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
		SPAIN
		ITALY
		SPAIN
		ITALY
		USA
		CANADA
		ITALY
		Germany
		Germany
		SWITZERLAND
		ITALY
		BELGIUM
		Germany
		Germany
		Germany
		HUNGARY
		GREAT BRITAIN
		NETHERLANDS
		SPAIN
		GREECE
		AUSTRIA
		AUSTRIA
		USA
		SPAIN
		SLOVAKIA
		Germany
		CANADA

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

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

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