Evaluation Report
proficiency test

DLA ptAL03 (2020)

Allergens III:

β-Lactoglobulin, Casein und Gluten

in Infant Food

DLA - Proficiency Tests GmbH
Kalte Weide 21
24641 Sievershütten/Germany

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

Coordinator of this PT:
Matthias Besler-Scharf, Ph.D./ Alexandra Scharf MSc.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptAL03 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf / Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (20. Juli 2020) 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: 20. Juli 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Content

1. Introduction....4
2. Realisation....4
2.1 Test material....4
2.1.1 Homogeneity....6
2.1.2 Stability....9
2.2 Sample shipment and information to the test....9
2.3 Submission of results....9
3. Evaluation....10
3.1 Consensus value from participants (assigned value)....10
3.2 Robust standard deviation....11
3.3 Exclusion of results and outliers....11
3.4 Target standard deviation (for proficiency assessment)....12
3.4.1 General model (Horwitz)....12
3.4.2 Value by precision experiment....12
3.4.3 Value by perception....15
3.5 z-Score....16
3.5.1 Warning and action signals....16
3.6 z'-Score....17
3.7 Quotient S*/σpt....17
3.8 Standard uncertainty and traceability....17
3.9 Figures of assigned values....18
3.10 Recovery rates: Spiking....18
4. Results....19
4.1 Proficiency Test Milk....21
4.1.1 ELISA Results: β-Lactoglobulin....21
4.1.2 ELISA Results: Casein....31
4.1.3 ELISA Results: Milk (as total milk protein)....41
4.1.4 PCR Results: Milk....48
4.2 Proficiency Test Wheat (Gluten)....49
4.2.1 ELISA Results: Gluten....49
4.2.2 PCR Results: Gluten....59
4.3 Participant z-Scores: overview table....60
5. Documentation....63
5.1 Details by the participants....63
5.1.1 ELISA: β-Lactoglobulin....63
5.1.2 ELISA: Casein....65
5.1.3 ELISA: Milk....66
5.1.4 ELISA: Gluten....67
5.1.5 PCR: Milk....69
5.1.6 PCR: Gluten....69
5.2 Homogeneity....70
5.2.1 Mixture homogeneity before bottling....70
5.3 Information on the Proficiency Test (PT)....71
6. Index of participant laboratories....72
7. Index of references....73

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 common in commerce infant food "cereal pap" from 4th month on (labeled as milk-free and gluten-free). The basic composition of both sample A and sample B was the same (see table 1).
After crushing and sieving by means of an impact mill (mesh 1,5 mm) the basic mixture was homogenized and an aliquot for sample A and sample B was taken.
Afterwards the **spiked sample A** 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. The prepared pap samples A and B were then each prepared according to the manufacturer's instructions with stirring and addition of water heated to 50°C.
The **spiking level sample** was produced with the allergenic compounds above mentioned by multi-stage addition of potato powder (mesh 500 µm) and homogenization.

The samples A and B were portioned to approximately 25 g into plastic containers, the spiking levels sample to approximately to 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 4th month Ingredients: Whole rice flour 32%, corn flour 30%, millet whole wheat flour 23%, whole buckwheat flour 15%, thiamine and preservative: sorbic acid Nutrients per 100 g powder: Fat 2,8 g, Carbohydrates 79 g, Protein 9,4 g	8,99 g/100 g	9,87 g/100g	-
Water	90,8 g/100g	90,1 g/100g	
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***	 66,3 mg/kg 21,9 mg/kg 17,5 mg/kg 2,19 mg/kg	-	 66,6 mg/kg 22,0 mg/kg 17,6 mg/kg 2,20 mg/kg
Milk component 2: whey powder mixture (4 products from Germany) - as whey powder * - thereof 15,9% total protein** - thereof β-Lactoglobulin***	 331 mg/kg 52,6 mg/kg 26,1 mg/kg	-	 343 mg/kg 54,4 mg/kg 27,2 mg/kg
Sum of milk components - thereof total protein - thereof Casein - *thereof β-Lactoglobulin*	*397 mg/kg* 74,5 mg/kg *17,5 mg/kg* *28,3 mg/kg*	-	*410 mg/kg* 76,4 mg/kg *17,6 mg/kg* *29,4 mg/kg*
Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) - as wheat flour* - thereof 10,1% total protein** - thereof gluten***	 254 mg/kg 25,7 mg/kg 22,1 mg/kg	-	 229 mg/kg 23,1 mg/kg 19,9 mg/kg
further Ingredients: *Maltodextrin, sodium sulfate and silicon dioxide*	<0,02 g/100 g	-	<0,02 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=6,38 for milk protein and F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 80% casein and 10% β-lactoglobulin in total milk protein [36]; approx. 50% approx. β-Lactoglobulin in whey powder [31]; 8,7% gluten in wheat flour [37, 38])

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 spiking level sample showed a probability of 84%. 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 0,8. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis. The sample weights were made with a deviation of ± 10% from recommended sample weight of the test kit instructions and not communicated to the laboratories. After transmission of analysis results by the laboratories, the valid results were calculated on the basis of the exact weightings by DLA and the statistical calculation was carried out according to ISO 13528:2015 Annex B (possibly with Notes 1 and 2).

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample A by all ELISA tests for milk protein and β-lactoglobulin (Immunolab and AgraQuant) as well as for gluten/gliadin (Immunolab) (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].

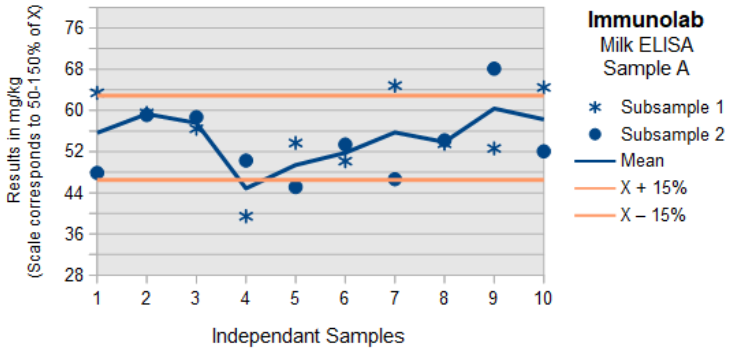
ELISA-Tests: Homogenität Milch / Homogeneity Milk

Immunolab Milkprotein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Milkprotein 54,7 ± 3,0 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	63,4	47,9	55,6
2	59,5	59,1	59,3
3	56,4	58,7	57,6
4	39,4	50,3	44,8
5	53,7	45,1	49,4
6	50,1	53,4	51,8
7	64,8	46,6	55,7
8	53,5	54,2	53,9
9	52,6	68,1	60,4
10	64,5	52,1	58,3

General average X	54,7	
SD of sample means Sx	4,84	8,8%
SD within-samples Sw	5,41	9,9%
SD between-samples Ss	2,96	5,4%



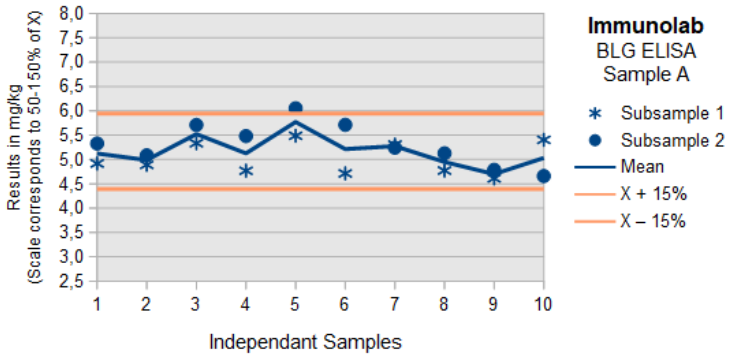
ELISA-Tests: Homogenität β-Lactoglobulin / Homogeneity β-Lactoglobulin

Immunolab β-Lactoglobulin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: β-Lactoglobulin 5,17 ± 0,24 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	4,92	5,33	5,12
2	4,89	5,09	4,99
3	5,33	5,71	5,52
4	4,77	5,48	5,12
5	5,49	6,05	5,77
6	4,71	5,71	5,21
7	5,31	5,24	5,27
8	4,77	5,13	4,95
9	4,61	4,79	4,70
10	5,40	4,66	5,03

General average X	5,17	
SD of sample means Sx	0,30	5,8%
SD within-samples Sw	0,27	5,2%
SD between-samples Ss	0,24	4,5%

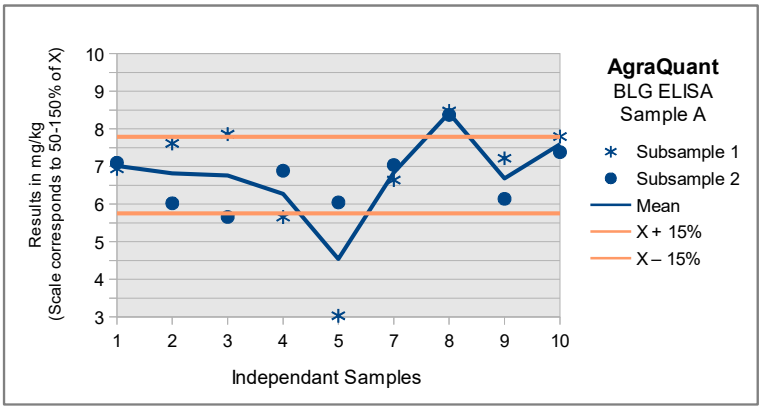


AgraQuant β-Lactoglobulin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: β-Lactoglobulin 6,77 ± 0,90 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,94	7,10	7,02
2	7,61	6,02	6,82
3	7,86	5,66	6,76
4	5,65	6,89	6,27
5	3,03	6,04	4,54
7	6,64	7,04	6,84
8	8,47	8,37	8,42
9	7,22	6,14	6,68
10	7,79	7,38	7,59

General average X	6,77	
SD of sample means Sx	1,04	15,4%
SD within-samples Sw	0,74	10,9%
SD between-samples Ss	0,90	13,4%



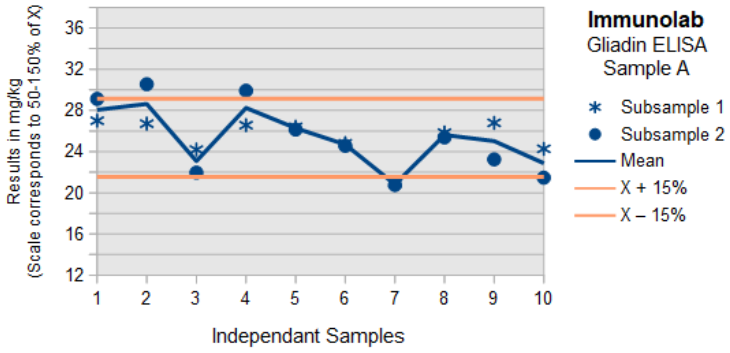
ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gliadin 25,3 ± 2,4 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	27,0	29,1	28,1
2	26,7	30,5	28,6
3	24,2	22,0	23,1
4	26,6	29,9	28,2
5	26,4	26,1	26,3
6	24,8	24,6	24,7
7	20,9	20,8	20,8
8	25,9	25,4	25,6
9	26,8	23,2	25,0
10	24,3	21,5	22,9

General average X	25,3	
SD of sample means Sx	2,58	10,2%
SD within-samples Sw	1,19	4,7%
SD between-samples Ss	2,44	9,6%



2.1.2 Stability

The pap samples are preparations preserved with sorbic acid. The stability of the sample material was thus guaranteed during the investigation period under the specified storage conditions.
A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,35 (17,9°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 2020. The testing method was optional. The tests should be finished at 22nd May 2020 the latest (extended).
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 range of mg/kg in the matrix of **Infant Food** (prepared pap, heated). One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "**spiking level sample**" contains the allergens in a simple matrix in **similar amounts** without further processing and should be analysed like a normal sample.*
Note: Please store the samples at 2-10°C on arrival.

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.

18 of 19 participants submitted their results in time. One participant submitted no 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 level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD	RSD_r	RSD_R	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]**	**Recovery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soya flour	Boiled sausage (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled sausage (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59
Wheat + Rye	Boiled sausage (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

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

The calculation is performed by:

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

If carried out an evaluation of the results by means of z'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.
The requirements for the analytical performance are generally considered as fulfilled if

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

If $U_{(xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.
The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

ELISA-results given as **gliadin** were converted into **gluten** multiplying the gliadin-content with the factor of 2.
A milk protein-specific ELISA result given as **skimmed milk powder** (method ELISA Fast, ifp) was converted to **total milk protein.** For this a milk protein content of 33% in skimmed milk powder was taken (see p. 5).

In the present PT all other ELISA results were submitted uniformly as casein oder β-lactoglobulin, 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 (Xpt)	$\boldsymbol{Xpt_{ALL}}$	$\boldsymbol{Xpt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (Xpt)		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σpt *or* $\sigma pt'$		
lower limit of target range ($Xpt - 2\sigma pt$) or ($Xpt - 2\sigma pt'$)°		
upper limit of target range ($Xpt + 2\sigma pt$) or ($Xpt + 2\sigma pt'$)°		
Quotient S*/σpt *or* S*/$\sigma pt'$		
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 Milk

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		
5a	positive	>400	negative	<0,01	2/2 (100%)	AQ	
16a	positive	23,2	negative	0	2/2 (100%)	EF	
13	positive	>1	negative	<0,1	2/2 (100%)	ES	
16b	positive		negative		2/2 (100%)	IF	Lateral Flow
3	positive	5,70	negative	<0,01	2/2 (100%)	IL	
4	positive	13,0	negative	<0,031	2/2 (100%)	MI-II	
2	positive	9,53	negative		2/2 (100%)	RS	
17	positive	43,6	negative	<	2/2 (100%)	RS	
5b	positive	>4,5	negative	<0,17	2/2 (100%)	RS-F	
6	positive	27,5	negative	<LOD	2/2 (100%)	RS-F	
7	positive	17,0	negative	<0,5	2/2 (100%)	RS-F	
8	positive	16,2	negative		2/2 (100%)	RS-F	
9	positive	20,5	negative		2/2 (100%)	RS-F	
12	positive	20,7	negative	<0,167	2/2 (100%)	RS-F	
14	positive	18,9	negative	<0,2	2/2 (100%)	RS-F	
15	positive	22,2	negative	<0,167	2/2 (100%)	RS-F	
18	positive	5,40	negative	<0,01	2/2 (100%)	SP	

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

Methods:

AQ = AgraQuant, RomerLabs
EF = ELISAFast, ipf
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ipf
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	β-Lactoglo-bulin	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
5a	>400			AQ	
16a	23,2	1,2		EF	
13	>1			ES	
16b				IF	
3	5,70	-2,7		IL	
4	13,0	-1,1		MI-II	
2	9,53	-1,9		RS	
17	43,6	5,8		RS	
5b	>4,5			RS-F	
6	27,5	2,2	1,5	RS-F	
7	17,0	-0,17	-0,63	RS-F	
8	16,2	-0,36	-0,79	RS-F	
9	20,5	0,62	0,07	RS-F	
12	20,7	0,66	0,11	RS-F	
14	18,9	0,26	-0,25	RS-F	
15	22,2	1,0	0,41	RS-F	
18	5,40	-2,8		SP	

Methods:

AQ = AgraQuant, RomerLabs
EF = ELISAFast, ipf
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ipf
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

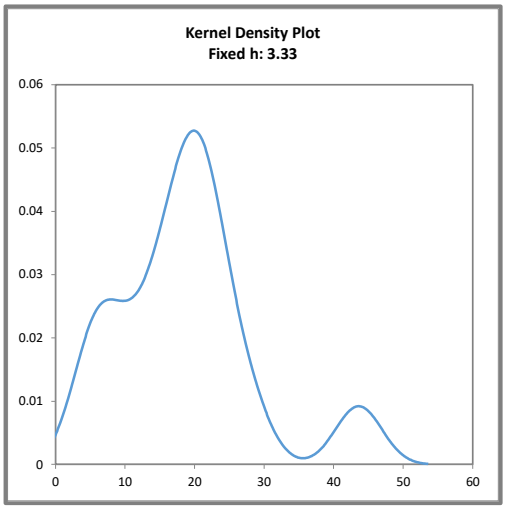


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a shoulder at approx. 8 mg/kg (several methods) and a secondary peak at approx. 44 mg/kg, due to a single value outside the target range.

Characteristics: Quantitative evaluation ELISA β-Lactoglobulin

Sample A

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	7
Number of outliers	0	0
Mean	18,7	20,4
Median	18,9	20,5
Robust Mean (X_{pt})	**17,8**	**20,2**
Robust standard deviation (S*)	**8,84**	**3,65**
Target range:		
Target standard deviation σ_{pt}	**4,44**	**5,04**
lower limit of target range	**8,88**	**10,1**
upper limit of target range	**26,6**	**30,2**
Quotient S/σ_{pt}*	*2,0*	*0,73*
Standard uncertainty $U_{(Xpt)}$	*3,07*	*1,73*
Results in the target range	*9*	*7*
Percent in the target range	*69*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no method-dependent differences.

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

The robust means of the evaluations were 63% and 71% of the spiking level of β-lactoglobulin to sample A and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates with z-scores ELISA for β-lactoglobulin ").

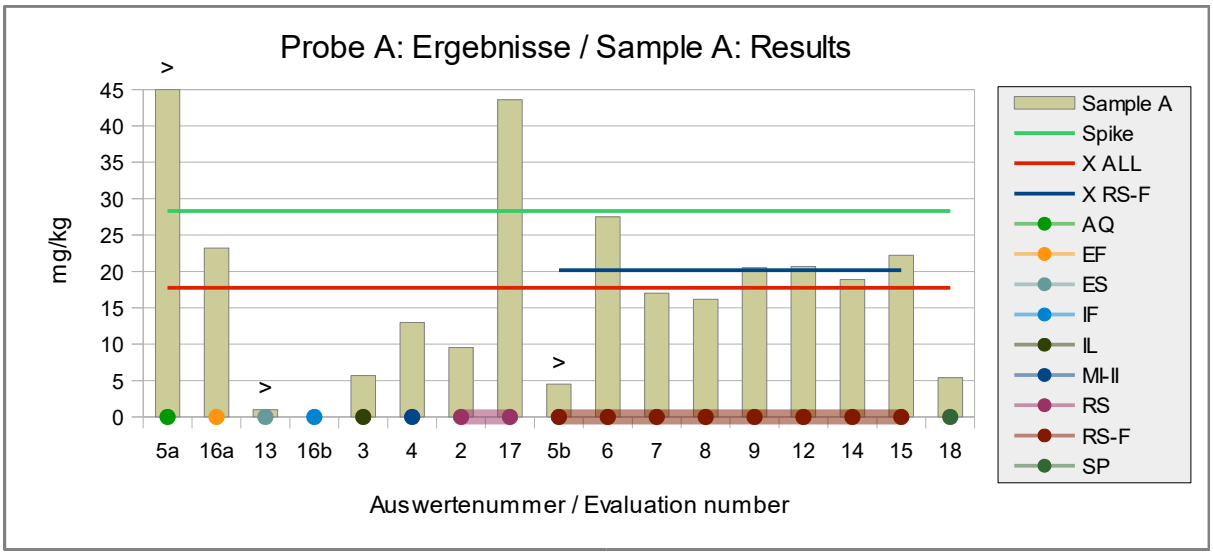


Abb./Fig. 2: ELISA Results β-Lactoblobulin
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

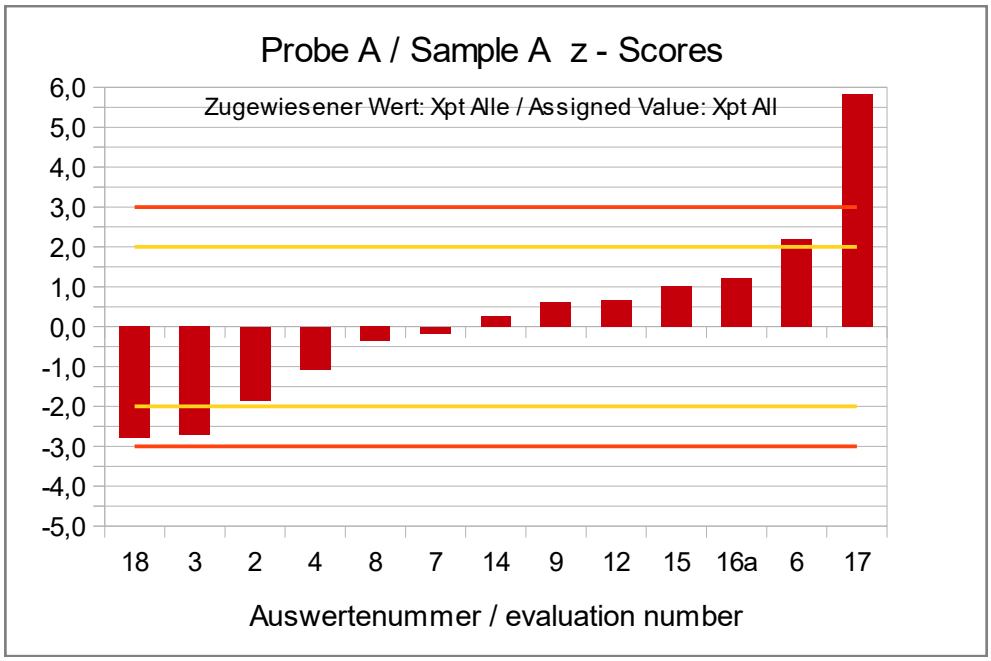


Abb./Fig. 3:
z-Scores ELISA Results β-Lactoblobulin
Assigned value robust mean of all results

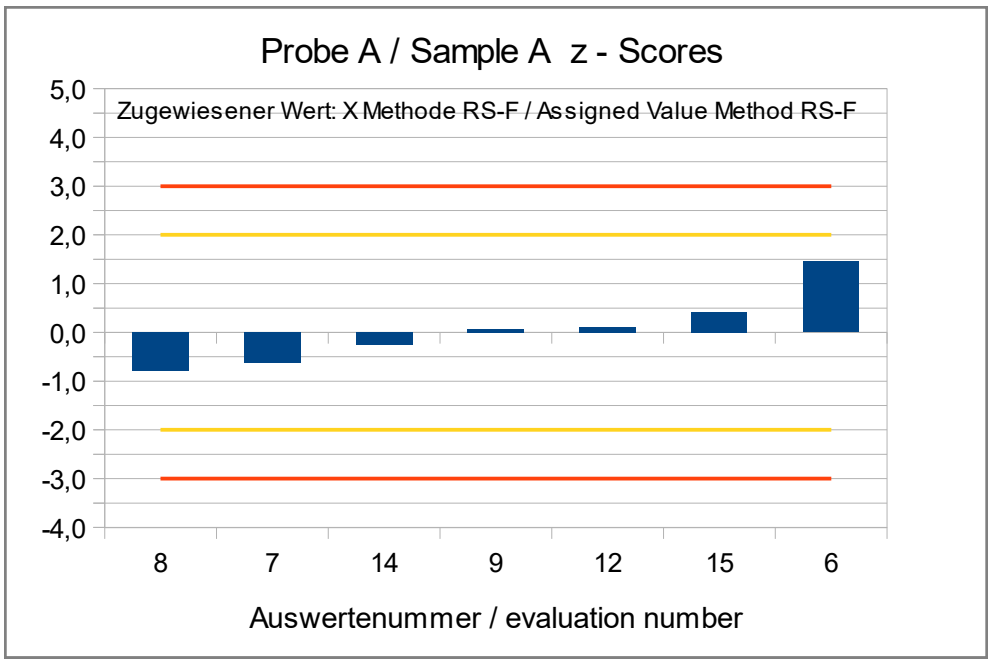


Abb./Fig. 4:
z-Scores ELISA Results β-Lactoblobulin, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	β-Lactoglo-bulin	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
5a	>400			AQ	
16a	24,4	2,0		EF	
13	>1			ES	
16b				IF	
3	6,50	-2,4		IL	
4	17,0	0,19		MI-II	
2	12,4	-0,94		RS	
17	24,8	2,1		RS	
5b	>4,5			RS-F	
6	22,4	1,5	1,3	RS-F	
7	28,0	2,9	2,6	RS-F	
8	8,76	-1,8	-1,9	RS-F	
9	10,0	-1,5	-1,7	RS-F	
12	15,7	-0,13	-0,32	RS-F	
14	16,1	-0,03	-0,22	RS-F	
15	18,4	0,53	0,31	RS-F	
18	6,60	-2,4		SP	

Methods:

AQ = AgraQuant, RomerLabs
EF = ELISAFast, ipf
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ipf
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

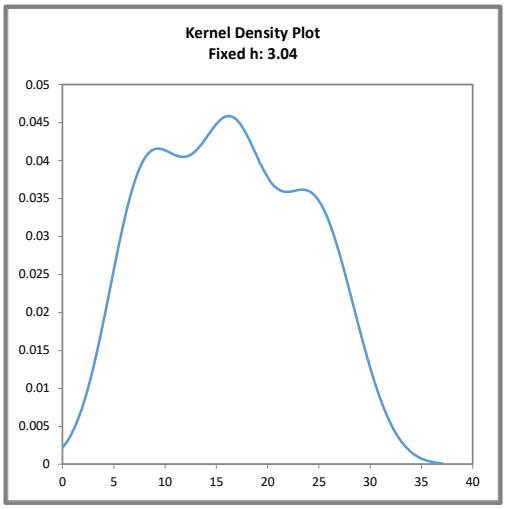


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows a wide distribution of results with two secondary maxima at approx. 9 mg/kg and at approx. 24 mg/kg next to the mean maximum at approx. 17 mg/kg. Method-dependent differences are not recognizable.

Characteristics: Quantitative evaluation ELISA β-Lactoblobulin

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	13	7
Number of outliers	0	0
Mean	16,2	17,1
Median	16,1	16,1
Robust Mean (X_{pt})	**16,2**	**17,1**
Robust standard deviation (S*)	**8,14**	**7,63**
Target range:		
Target standard deviation σ_{pt}	**4,06**	**4,26**
lower limit of target range	**8,12**	**8,53**
upper limit of target range	**24,4**	**26**
Quotient S/σ_{pt}*	*2,0*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*2,82*	*3,61*
Results in the target range	*8*	*6*
Percent in the target range	*62*	*86*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a broad distribution without clear method-dependent differences.

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

The robust means of the evaluations were 55% and 58% of the spiking level of β-lactoglobulin to the spiking level sample and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for β-lactoglobulin").

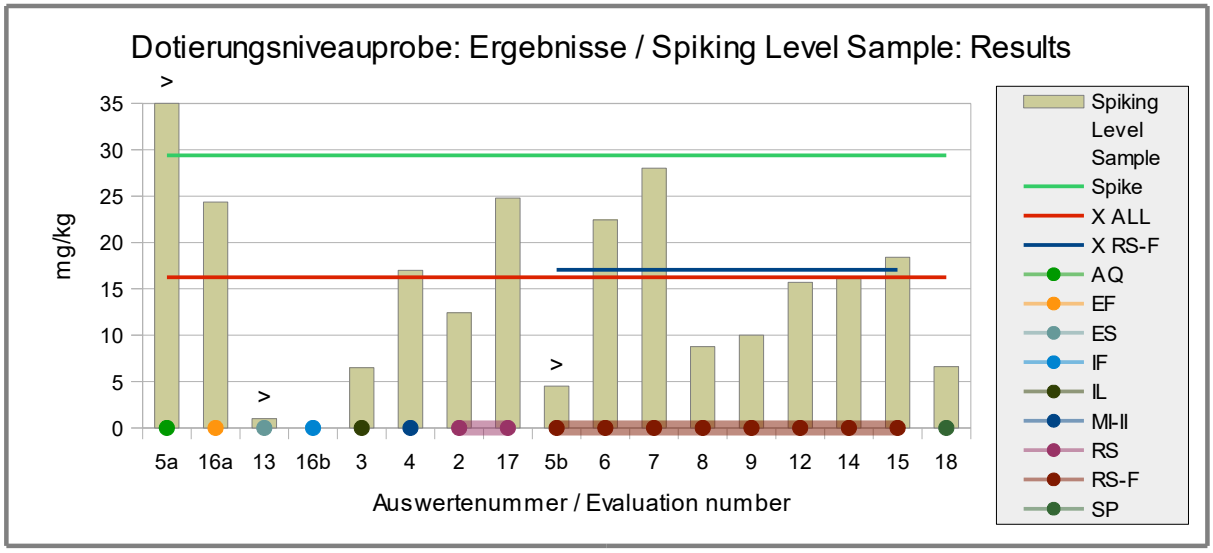


Abb./Fig. 6: ELISA Results β-Lactoglobulin
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

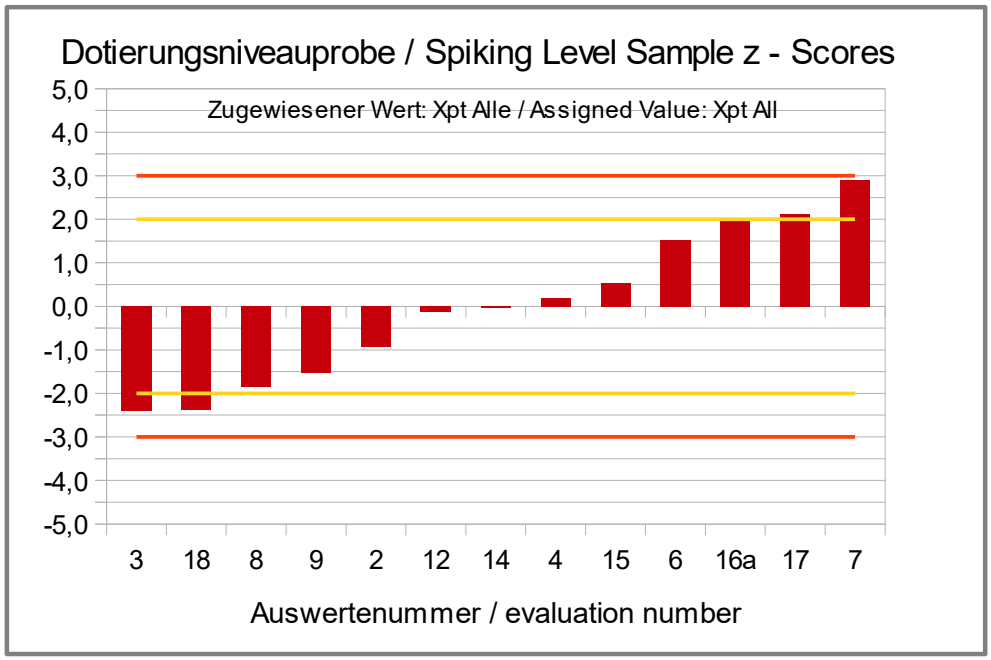


Abb./Fig. 7:
z-Scores ELISA Results β-Lactoglobulin
Assigned value robust mean of all results

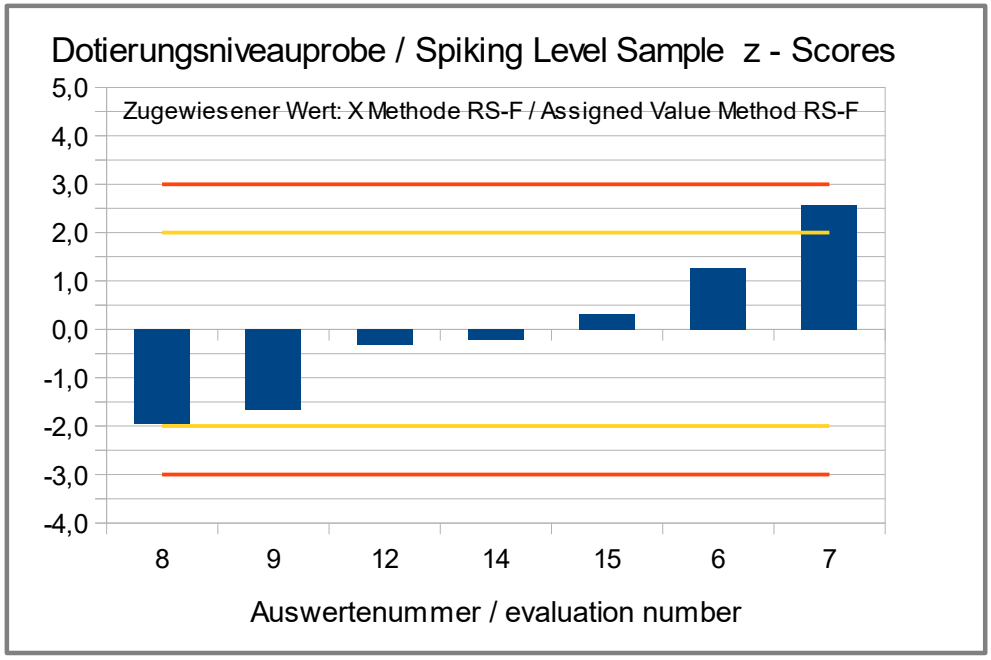


Abb./Fig. 8:
z-Scores ELISA Results β-Lactoglobulin Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
5a	>400			>400			AQ	
16a	24,4	**83**	-0,69	23,2	**82**	-0,72	EF	
13	>1			>1			ES	
16b							IF	
3	6,50	22	-3,1	5,70	20	-3,2	IL	
4	17,0	**58**	-1,7	13,0	46	-2,2	MI-II	
2	12,4	42	-2,3	9,53	34	-2,7	RS	
17	24,8	**84**	-0,63	43,6	154	2,2	RS	
5b	>4,5			>4,5			RS-F	
6	22,4	**76**	-0,95	27,5	**97**	-0,11	RS-F	
7	28,0	**95**	-0,19	17,0	**60**	-1,6	RS-F	
8	8,76	30	-2,8	16,2	**57**	-1,7	RS-F	
9	10,0	34	-2,6	20,5	**72**	-1,1	RS-F	
12	15,7	**53**	-1,9	20,7	**73**	-1,1	RS-F	
14	16,1	**55**	-1,8	18,9	**67**	-1,3	RS-F	
15	18,4	**63**	-1,5	22,2	**79**	-0,9	RS-F	
18	6,60	22	-3,1	5,40	19	-3,2	SP	

RA**	50-150 %	RA**	50-150 %
Number in RA	8	Number in RA	8
Percent in RA	62	Percent in RA	62

* Recovery rate 100% relative size: β-lactoglobulin, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
EF = ELISAFast, ipf
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ipf
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comment:

62% (8) of the participants obtained for the spiking level sample and for the processed spiked food matrix sample A a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%.
The related z-scores are based on the target standard deviation of 25%.

4.1.2 ELISA Results: Casein

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
5a	positive	8,40	negative	< 1	2/2 (100%)	AQ	
9	positive	14,8	negative		2/2 (100%)	AQ	
11	positive	13,2	negative	<0,2	2/2 (100%)	AQ	
10	positive	11,3	negative	<LOQ	2/2 (100%)	BF	
16a	positive	8,69	negative	0	2/2 (100%)	EF	
13	positive	>10	negative	<1	2/2 (100%)	ES	
17	positive	8,80	negative	<	2/2 (100%)	ES	
16b	positive		negative		2/2 (100%)	IF	Lateral Flow
3	positive	4,90	negative	<0,2	2/2 (100%)	IL	
4	positive	18,0	negative	<0,25	2/2 (100%)	MI-II	
5b	positive	10,5	negative	< 0,5	2/2 (100%)	RS-F	
6	positive	21,0	negative	<LOD	2/2 (100%)	RS-F	
7	positive	15,0	negative	<2,5	2/2 (100%)	RS-F	
8	positive	15,9	negative		2/2 (100%)	RS-F	
12	positive	23,6	negative	<2,5	2/2 (100%)	RS-F	
14	positive	18,2	negative	<0,71	2/2 (100%)	RS-F	
18	positive	30,0	negative	<0,1	2/2 (100%)	SP	

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

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Casein	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
5a	8,40	-1,7		AQ	
9	14,8	0,10		AQ	
11	13,2	-0,35		AQ	
10	11,3	-0,87		BF	
16a	8,69	-1,6		EF	
13	>10			ES	
17	8,80	-1,6		ES	
16b				IF	
3	4,90	-2,6		IL	Lateral Flow
4	18,0	0,99		MI-II	
5b	10,5	-1,1	-1,6	RS-F	
6	21,0	1,8	0,84	RS-F	
7	15,0	0,16	-0,54	RS-F	
8	15,9	0,40	-0,34	RS-F	
12	23,6	2,5	1,4	RS-F	
14	18,2	1,0	0,19	RS-F	
18	30,0	4,3		SP	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

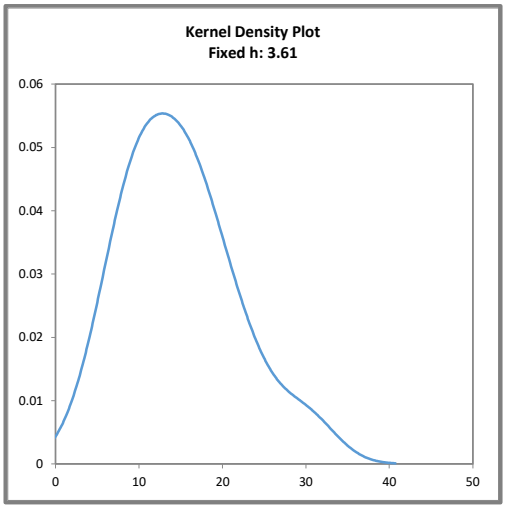


Abb. / Fig. 9:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a slight shoulder at >25 mg/kg.

Characteristics: Quantitative evaluation ELISA Casein

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	15	6
Number of outliers	0	0
Mean	14,8	17,4
Median	14,8	17,0
Robust Mean (X_{pt})	**14,4**	**17,4**
Robust standard deviation (S*)	**6,59**	**5,26**
Target range:		
Target standard deviation σ_{pt}	**3,61**	**4,34**
lower limit of target range	**7,22**	**8,68**
upper limit of target range	**21,7**	**26,0**
Quotient S/σ_{pt}*	*1,8*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*2,13*	*2,69*
Results in the target range	*12*	*6*
Percent in the target range	*80*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed nearly a symmetrical distribution of results.

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

The robust means of the evaluations were 82% and 99% of the spiking level of casein to sample A and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.40 "Recovery rates with z-scores ELISA for casein").

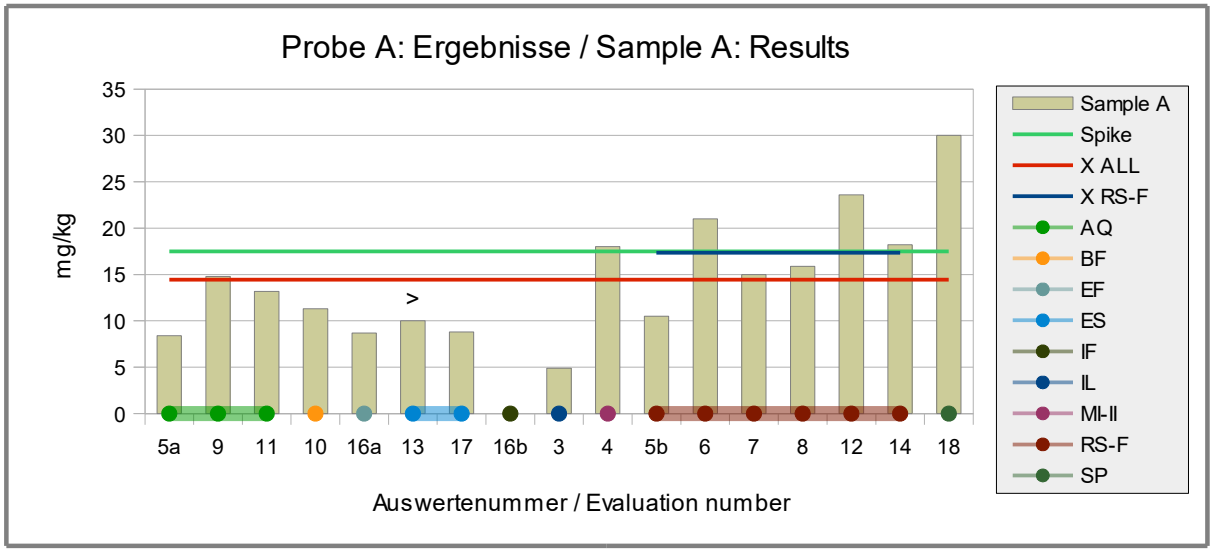


Abb./Fig. 10: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

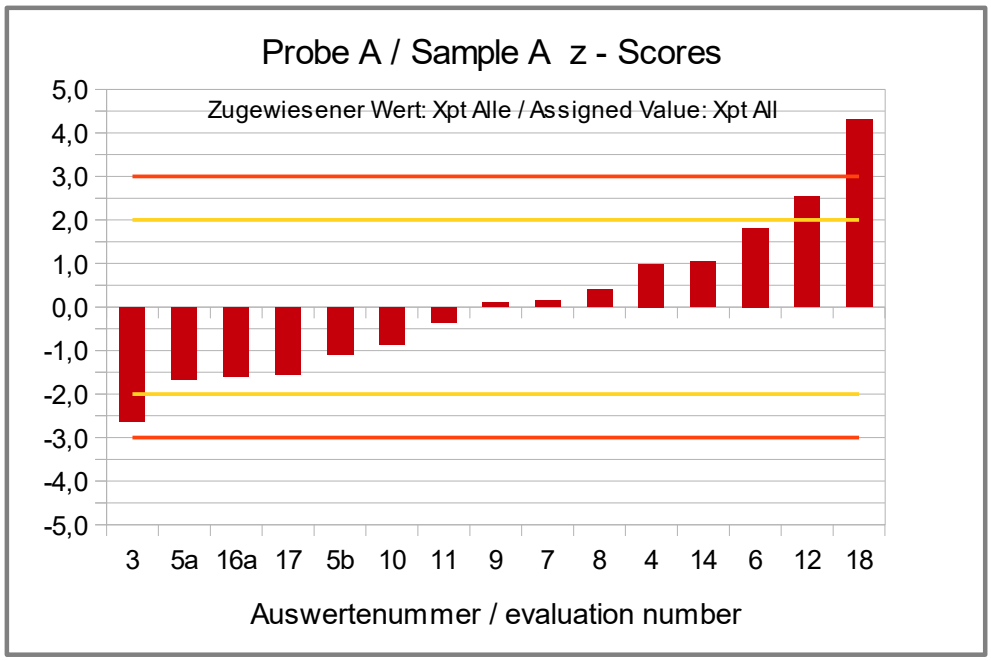


Abb./Fig. 11:
z-Scores ELISA Results Casein
Assigned value robust mean of all results

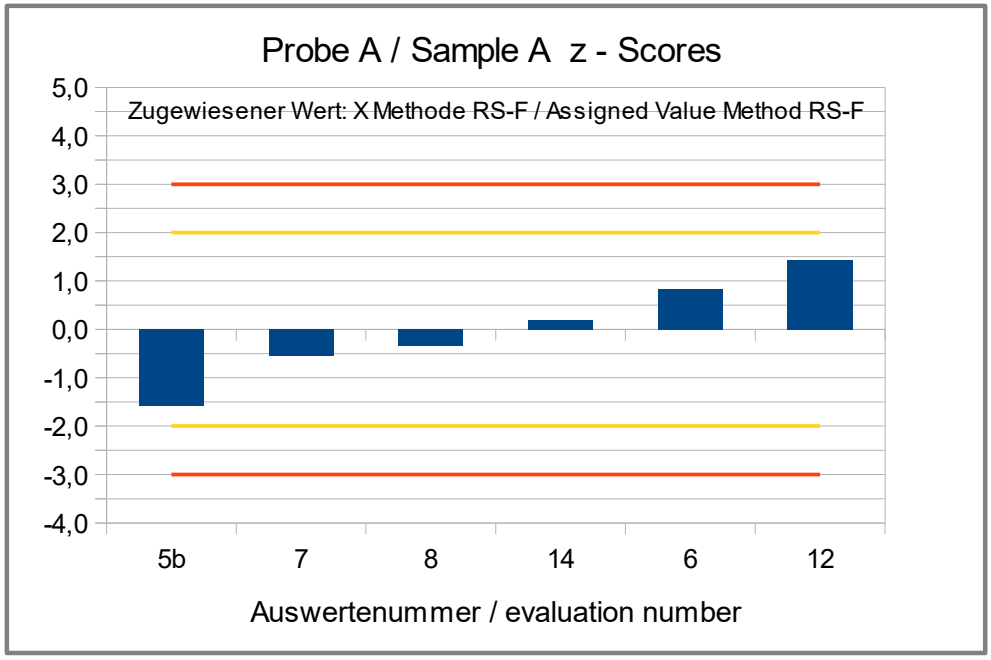


Abb./Fig. 12:
z-Scores ELISA Results Casein, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Casein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
5a	11,0	-1,7		AQ	
9	27,7	1,8		AQ	
11	30,3	2,3		AQ	
10	12,3	-1,4		BF	
16a	8,33	-2,3		EF	
13	8,30	-2,3		ES	
17	1,90	-3,6		ES	
16b				IF	
3	16,5	-0,56		IL	
4	20,0	0,17		MI-II	
5b	>13,5			RS-F	
6	27,7	1,8	0,78	RS-F	
7	27,0	1,6	0,66	RS-F	
8	19,9	0,16	-0,56	RS-F	
12	25,1	1,2	0,33	RS-F	
14	16,1	-0,64	-1,2	RS-F	
18	34,0	3,1		SP	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = ELISA Fast, ifp
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

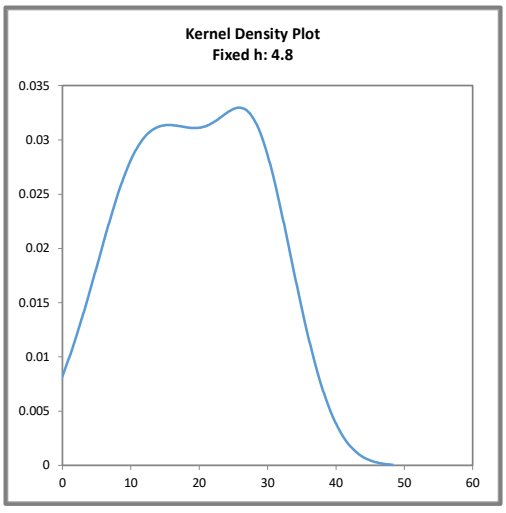


Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows a broad distribution of results with two maxima at approx. 15 mg/kg and at approx. 27 mg/kg. A method dependency is not recognizable.

Characteristics: Quantitative evaluation ELISA Casein

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	15	5
Number of outliers	0	0
Mean	19,1	23,2
Median	19,9	25,1
Robust Mean (X_{pt})	**19,2**	**23,2**
Robust standard deviation (S*)	**10,5**	**5,65**
Target range:		
Target standard deviation σ_{pt}	**4,80**	**5,79**
lower limit of target range	**9,59**	**11,6**
upper limit of target range	**28,8**	**34,8**
Quotient S/σ_{pt}*	*2,2*	*0,98*
Standard uncertainty $U_{(Xpt)}$	*3,39*	*3,16*
Results in the target range	*10*	*5*
Percent in the target range	*67*	*100*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a broad distribution with two maxima and without clear method-dependent differences.

The evaluation of the results of all methods showed a slightly increased variability and a low variability for method RS-F. The quotients S/σ_{pt} were at 2,2 and below 1,0. The robust standard deviations are above the range or in the range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 109% and 132% of the spiking level of casein to the spiking level sample and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.40 "Recovery rates ELISA for casein").

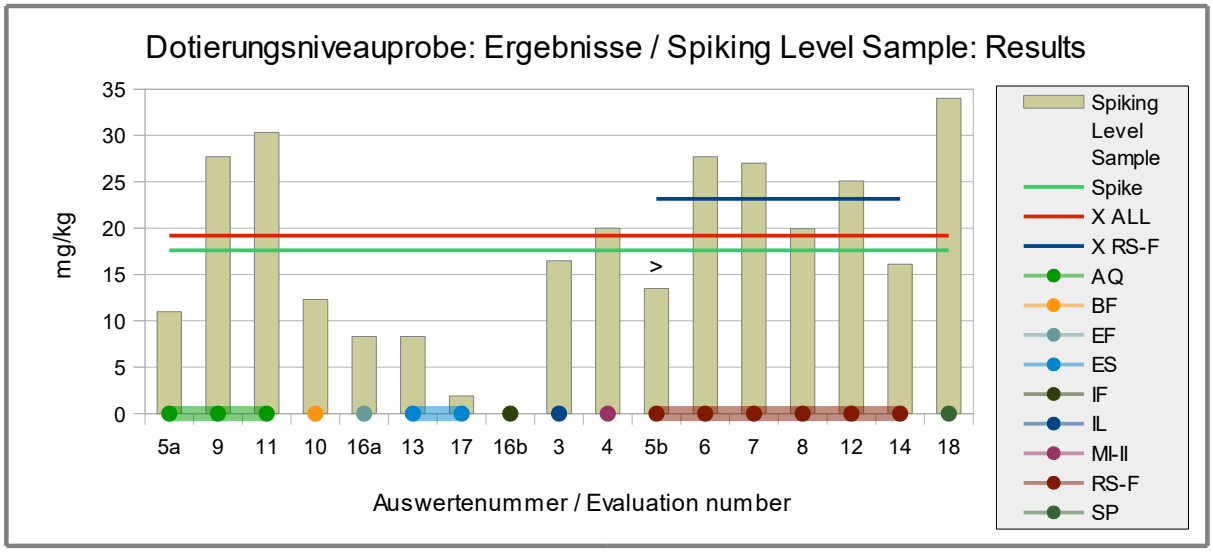


Abb./Fig. 14: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

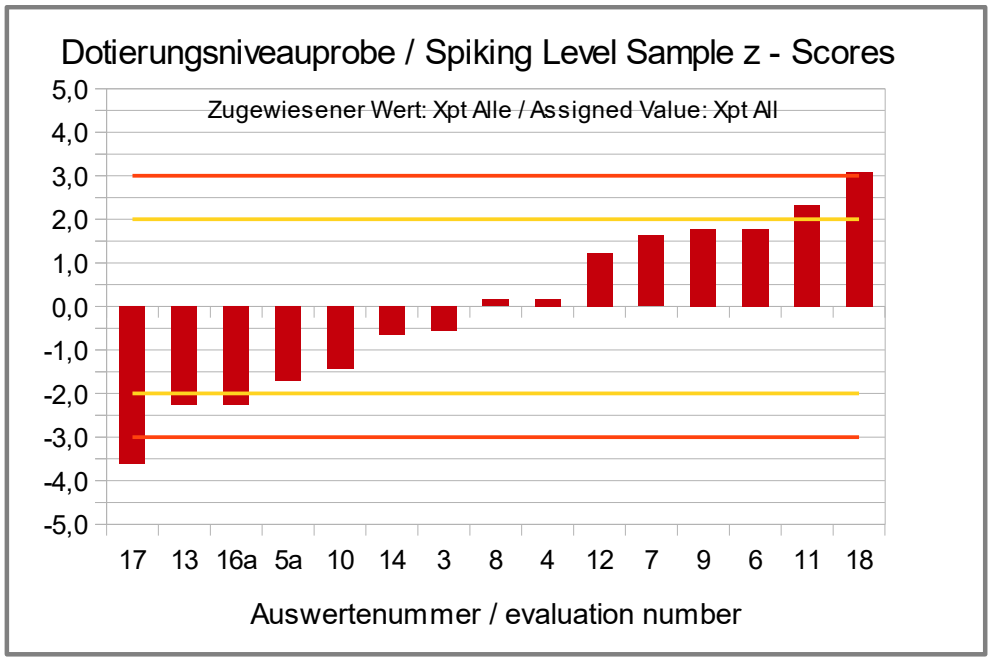


Abb./Fig. 15:
z-Scores ELISA Results Casein
Assigned value robust mean of all results

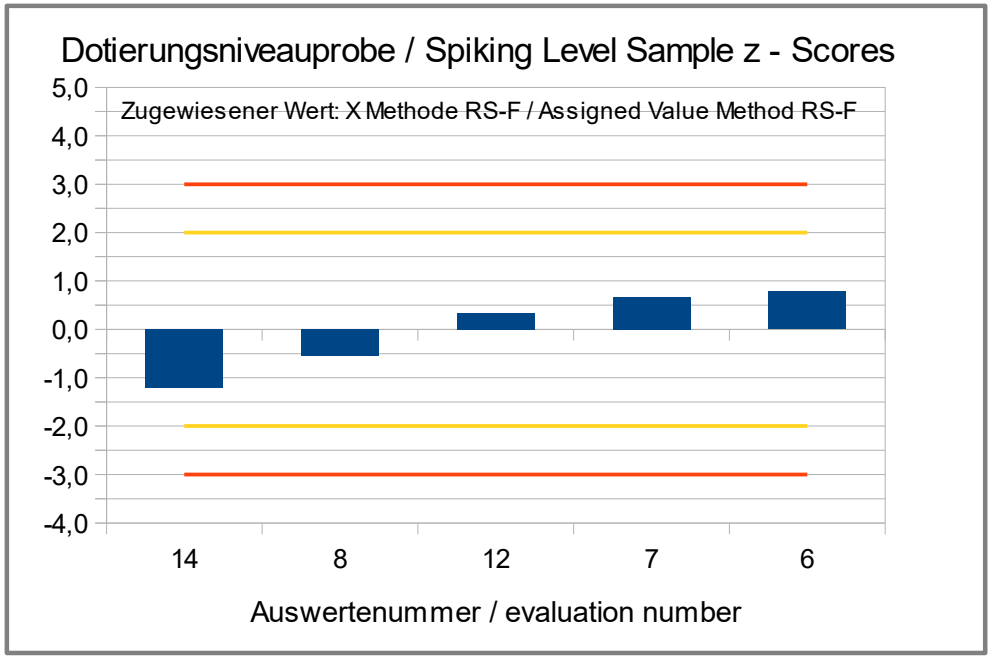


Abb./Fig. 16:
z-Scores ELISA Results Casein, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
5a	11,0	**63**	-1,5	8,40	48	-2,1	AQ	
9	27,7	157	2,3	14,8	**85**	-0,6	AQ	
11	30,3	172	2,9	13,2	**75**	-1,0	AQ	
10	12,3	**70**	-1,2	11,3	**65**	-1,4	BF	
16a	8,33	47	-2,1	8,69	**50**	-2,0	EF	
13	8,30	47	-2,1	>10			ES	
17	1,90	11	-3,6	8,80	**50**	-2,0	ES	
16b							IF	
3	16,5	**94**	-0,3	4,90	28	-2,9	IL	
4	20,0	**114**	0,55	18,0	**103**	0,11	MI-II	
5b	>13,5			10,5	**60**	-1,6	RS-F	
6	27,7	157	2,3	21,0	**120**	0,80	RS-F	
7	27,0	153	2,1	15,0	**86**	-0,6	RS-F	
8	19,9	**113**	0,53	15,9	**91**	-0,4	RS-F	
12	25,1	**143**	1,7	23,6	**135**	1,4	RS-F	
14	16,1	**91**	-0,3	18,2	**104**	0,16	RS-F	
18	34,0	193	3,7	30,0	171	2,9	SP	

RA**	50-150 %	RA**	50-150 %
Number in RA	7	Number in RA	12
Percent in RA	47	Percent in RA	80

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comment:

47% (7) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A 80% (12) of the obtained recovery rates were within the recommended range.
The related z-scores are based on the target standard deviation of 25%.

4.1.3 ELISA Results: Milk (as total 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		
16a	positive	246	negative	0	2/2 (100%)	ES	Result converted °
16b	positive		negative		2/2 (100%)	IF	Lateral Flow
6	positive	252	negative	<LOD	2/2 (100%)	RS-F	
8	positive	251	negative		2/2 (100%)	RS-F	
15	positive	279	negative	<2,5	2/2 (100%)	RS-F	
18	positive	34,0	negative	< 0.2	2/2 (100%)	SP	Results sum of casein and β-lactoglobulin

° calculation see p. 19

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

Methods:
ES = ELISA-Systems
IF = ImmunoFast (Lateral Flow), ifp
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA: Sample A *(informative)*

Evaluation number	Total milk protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
16a	246	-0,01	ES	Result converted °
16b			IF	
6	252	0,10	RS-F	
8	251	0,07	RS-F	
15	279	0,52	RS-F	
18	34,0	-3,4	SP	Results sum of casein and β-lactoglobulin

° calculation see p. 19

Methods:
ES = ELISA-Systems
IF = ImmunoFast, ifp
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comments:
Due to the small number of results, the evaluation was carried out for information only. A kernel density estimation was not made.

Characteristics: Quantitative evaluation ELISA Milk (as total milk protein) ***(informative)***

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	5
Number of outliers	-
Mean	212
Median	251
Robust Mean (X_{pt})	**247**
Robust standard deviation (S*)	**30,3**
Target range:	
Target standard deviation σ_{pt}	**61,6**
lower limit of target range	**123**
upper limit of target range	**370**
Quotient S/σ_{pt}*	*0,49*
Standard uncertainty $U_{(Xpt)}$	*17,0*
Results in the target range	*4*
Percent in the target range	*80*

Comments to the statistical characteristics and assigned values:

Due to the small number of results, the evaluation was carried out for information only.

The robust mean of the evaluation was 331% of the spiking level of total milk protein to sample A and was well above the range of the recommendations for the applied methods (s. 3.4.3 and p.47 "Recovery rates with z-scores ELISA for milk").

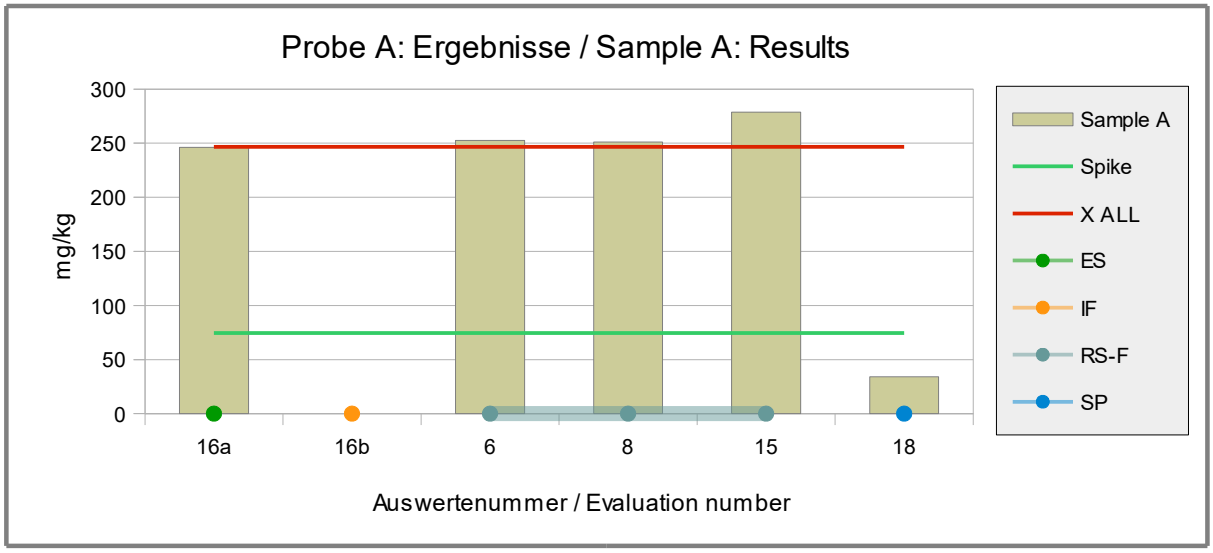


Abb./Fig. 17: ELISA Results Milk (as total milk protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

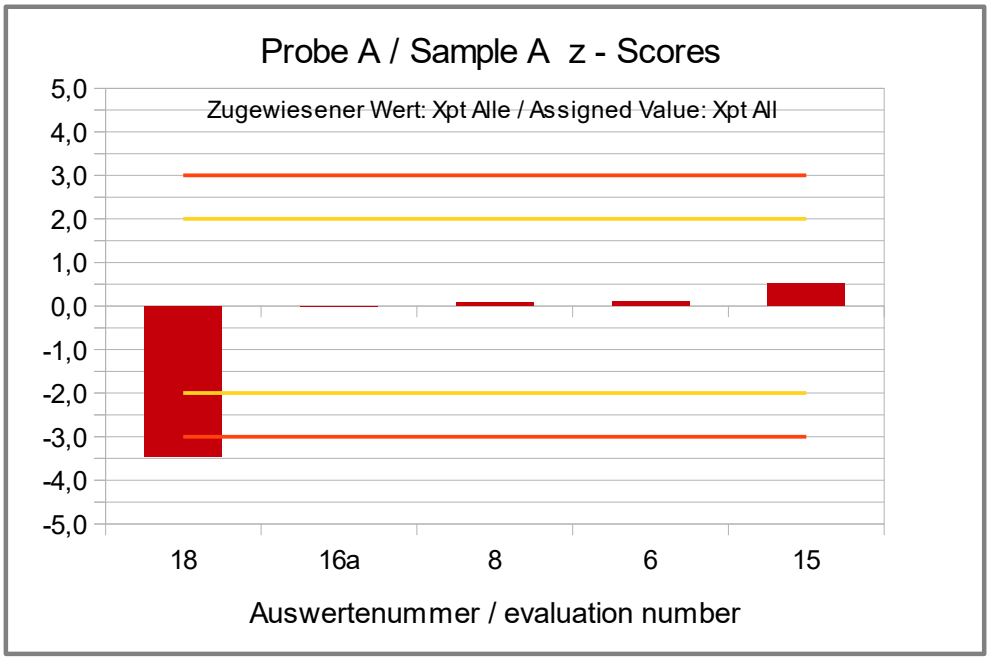


Abb./Fig. 18:
z-Scores ELISA Results Milk (as total milk protein)
Assigned value robust mean of all results

Quantitative valuation of ELISA: Spiking Level Sample *(informative)*

Evaluation number	Total milk protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
16a	252	0,90	ES	Result converted °
16b			IF	
6	214	0,16	RS-F	
8	176	-0,59	RS-F	
15	206	0,00	RS-F	
18	45,0	-3,1	SP	Results sum of casein and β-lactoglobulin

° calculation see p. 19

Methods:

ES = ELISA-Systems

IF = ImmunoFast, ifp

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comment:

Due to the small number of results, the evaluation was carried out for information only. A kernel density estimation was not made due to the number of <8 results.

Characteristics: Quantitative evaluation ELISA Milk (as total milk protein) *(informative)*

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	179
Robust Mean	184
Median (X_{pt})	**206**
Robust standard deviation (S*)	**76,9**
Target range:	
Target standard deviation σ_{pt}	**51,5**
lower limit of target range	**103**
upper limit of target range	**309**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*43,0*
Results in the target range	*4*
Percent in the target range	*80*

Comments to the statistical characteristics and assigned values:

Due to the small number of results, the evaluation was carried out for information only.

The median of the evaluation was 269% of the spiking level of total milk protein to the spiking level sample and was well above the range of the recommendations for the applied methods (s. 3.4.3 and p.47 "Recovery rates ELISA for milk").

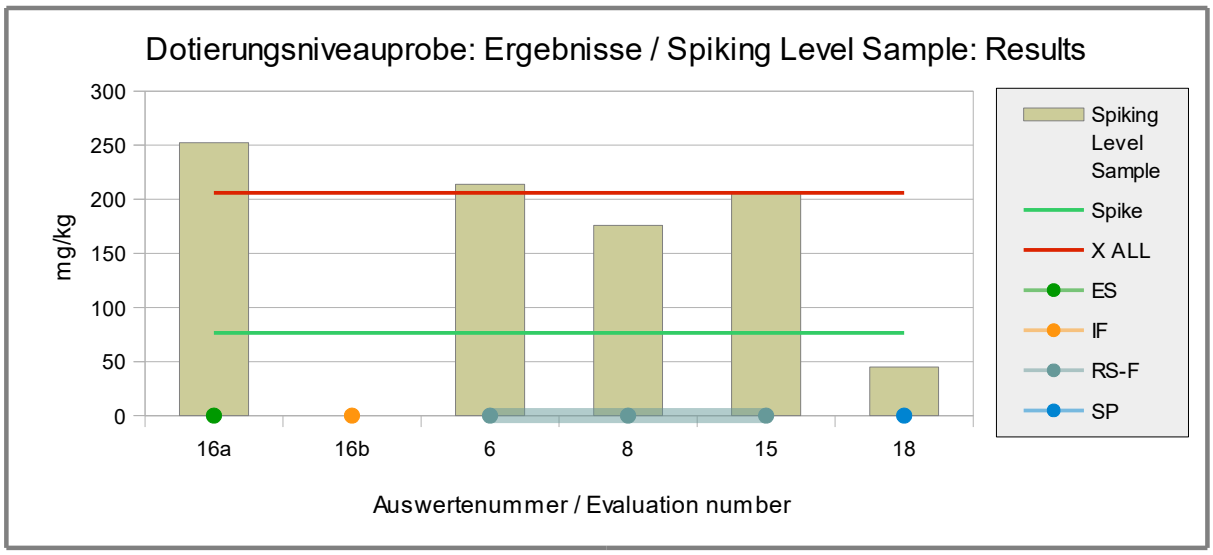


Abb./Fig. 19: ELISA Results Milk (as total milk protein)
green line = Spiking level (Spike)
red line = Assigned value median all results
round symbols = Applied methods (see legend)

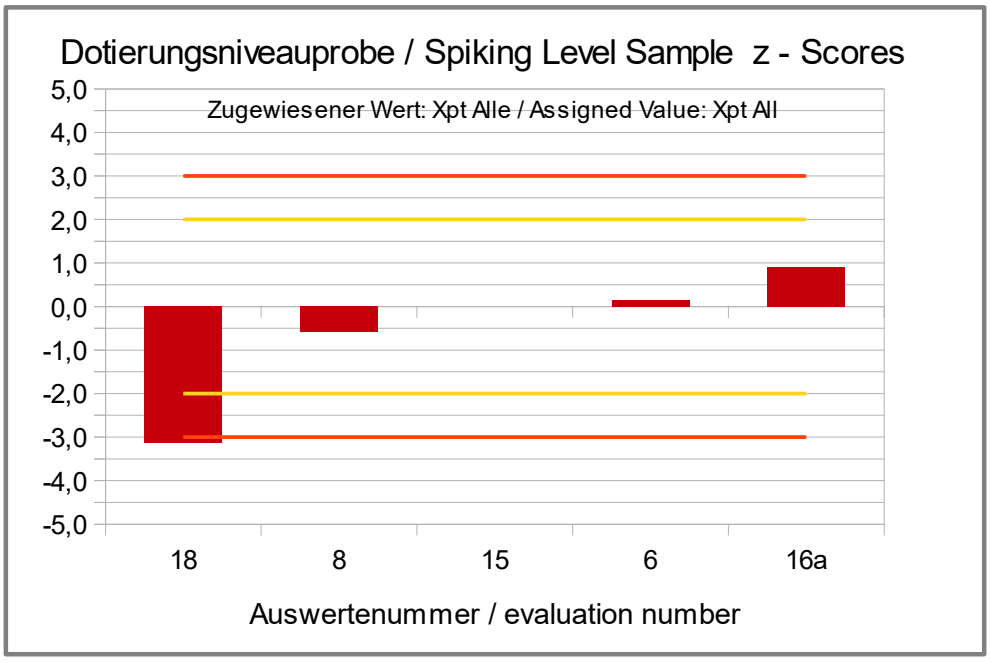


Abb./Fig. 20:
z-Scores ELISA Results milk (as total milk protein)
Assigned value median of all results

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
16a	252	330	9,2	246	330	9,2	ES	Result converted °
16b							IF	
6	214	280	7,2	252	339	9,6	RS-F	
8	176	230	5,2	251	337	9,5	RS-F	
15	206	269	6,8	279	374	11,0	RS-F	
18	45,0	**59**	-1,6	34,0	46	-2,2	SP	Results sum of casein and β-lactoglobulin

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**1**	Number in RA	**0**
Percent in RA	**20**	Percent in RA	**0**

Methods:
ES = ELISA-Systems
IF = ImmunoFast, ifp
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:

The recovery rates of 4 participants (methods ES and RS-F) were for the spiking level sample and the processed spiked food matrix sample A in the range of 230-330% or 330-374%, respectively. One participant obtained with method SP for both samples recovery rates of around 50%. The related z-scores are based on the target standard deviation of 25%.

It should be noted that the milk protein composition of the PT samples does not correspond to the natural ratio of casein to whey protein. The whey protein content is increased (s. page 5). Depending on the specificity of the methods used, this can lead to a changed response for total milk protein.

4.1.4 PCR Results: Milk

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
17	positive		negative		2/2 (100%)	div	Detection of cow DNA

	Sample A		Sample B	
Spiking	positive		negative	

Methods:
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Qualitative valuation of results: Spiking level sample

Evaluation number	Milk	Milk	Method	Remarks
	pos/neg	[mg/kg]		
17	positive		div	Detection of cow DNA

Spiking Level Sample	
Spiking	positive

Methods:
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

4.2 Proficiency Test Wheat (Gluten)

4.2.1 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		
10	positive	25,5	negative	<LOQ	2/2 (100%)	BF	
16a	positive	38,8	negative	0	2/2 (100%)	EF	
16b	positive		negative		2/2 (100%)	IF	Lateral Flow
3	positive	87,5	negative	<4	2/2 (100%)	IL	
18	positive	42,0	negative	<2	2/2 (100%)	IL	Result converted °
4a	positive	22,0	negative	<5	2/2 (100%)	RS	
5	positive	28,9	negative	<3	2/2 (100%)	RS	
7	positive	15,0	negative	<5	2/2 (100%)	RS	
8	positive	21,4	negative		2/2 (100%)	RS	
9	positive	27,7	negative		2/2 (100%)	RS	
11	positive	23,1	negative	<5	2/2 (100%)	RS	
12	positive	25,7	negative	<5	2/2 (100%)	RS	
13a	positive	14,0	negative	<5	2/2 (100%)	RS	
14	positive	16,5	negative	< 2,7	2/2 (100%)	RS	
15	positive	14,6	negative	<5	2/2 (100%)	RS	
17	positive	31,0	negative	<	2/2 (100%)	RS	
6	positive	25,9	negative	<LOD	2/2 (100%)	RS-F	
13b	positive	17,0	negative	<2,5	2/2 (100%)	RS-F	
4b	positive	22,0	negative	<3,12	2/2 (100%)	SP	
1	positive	14,3	negative	0	2/2 (100%)	VT	

° calculation see p. 19

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative valuation of ELISA results: Sample A

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
10	25,5	0,24		BF	
16a	38,8	2,4		EF	
16b				IF	
3	87,5	11		IL	
18	42,0	3,0		IL	Result converted °
4a	22,0	-0,34	0,04	RS	
5	28,9	0,80	1,3	RS	
7	15,0	-1,5	-1,2	RS	
8	21,4	-0,44	-0,07	RS	
9	27,7	0,60	1,1	RS	
11	23,1	-0,16	0,23	RS	
12	25,7	0,27	0,71	RS	
13a	14,0	-1,7	-1,4	RS	
14	16,5	-1,3	-0,97	RS	
15	14,6	-1,6	-1,3	RS	
17	31,0	1,2	1,7	RS	
6	25,9	0,31		RS-F	
13b	17,0	-1,2		RS-F	
4b	22,0	-0,34		SP	
1	14,3	-1,6		VT	

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

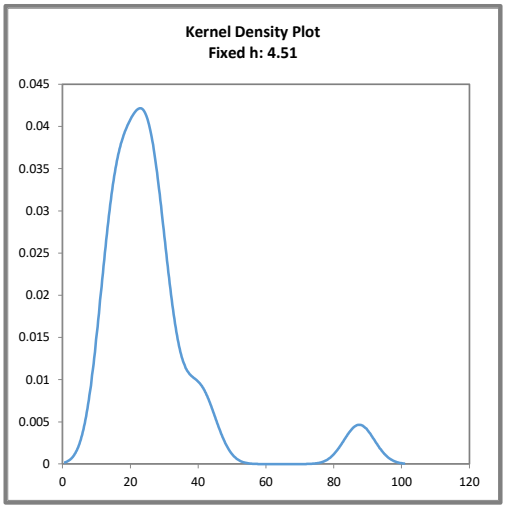


Abb. / Fig. 21:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetric distribution of results with a slight shoulder at approx. 40 mg/kg (method EF and IL) and a secondary peak at 88 mg/kg, due to a single result out of the target range (method IL).

Characteristics: Quantitative evaluation ELISA gluten

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	19	11
Number of outliers	-	0
Mean	27,0	21,8
Median	23,1	22,0
Robust Mean (X_{pt})	**24,1**	**21,8**
Robust standard deviation (S*)	**8,98**	**6,94**
Target range:		
Target standard deviation σ_{pt}	**6,02**	**5,45**
lower limit of target range	**12,0**	**10,9**
upper limit of target range	**36,1**	**32,7**
Quotient S/σ_{pt}*	*1,5*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*2,58*	*2,61*
Results in the target range	*16*	*11*
Percent in the target range	*84*	*100*

Method:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation shows nearly a symmetric distribution.

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

The robust means of the evaluations were 109% and 99% of the spiking level of gluten to sample A and in the range of the recommendations for the applied methods (s. 3.4.3 and p.58 "Recovery rates ELISA for gluten").

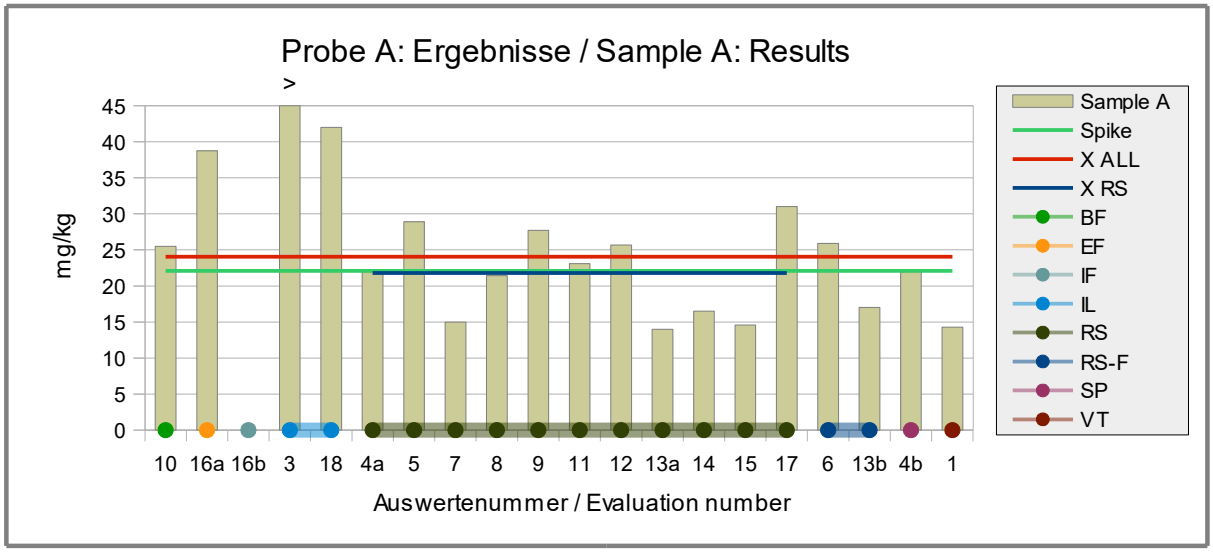


Abb./Fig. 22: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

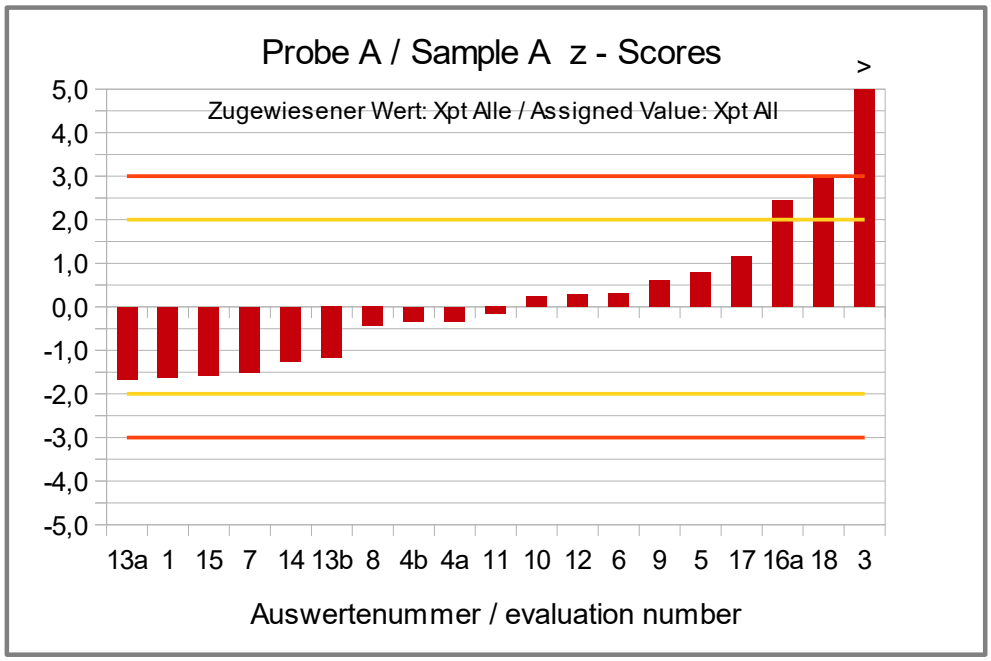


Abb./Fig. 23:
z-Scores ELISA Results Gluten
Assigned value median of all results

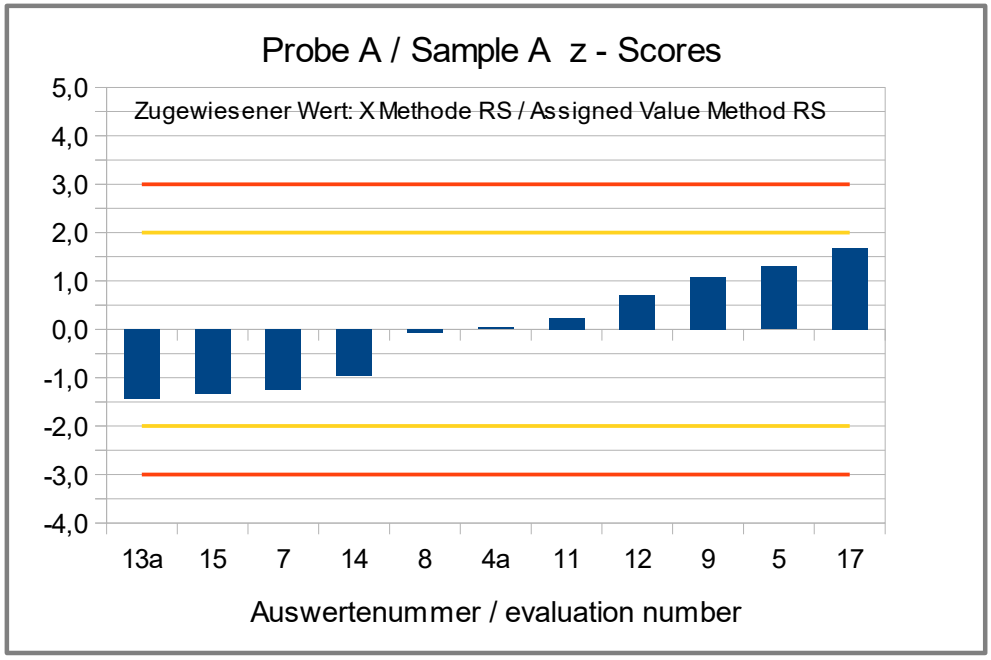


Abb./Fig. 24:
z-Scores ELISA Results Gluten Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
10	31,5	0,98		BF	
16a	34,6	1,5		EF	
16b				IF	
3	89,5	10		IL	
18	72,0	7,4		IL	Result converted °
4a	22,0	-0,53	-0,13	RS	
5	27,7	0,38	0,87	RS	
7	25,0	-0,05	0,40	RS	
8	19,0	-0,99	-0,65	RS	
9	24,0	-0,21	0,22	RS	
11	23,1	-0,35	0,07	RS	
12	26,5	0,19	0,66	RS	
13a	16,0	-1,5	-1,2	RS	
14	15,0	-1,6	-1,4	RS	
15	26,2	0,14	0,62	RS	
17	24,8	-0,08	0,36	RS	
6	31,1	0,91		RS-F	
13b	19,0	-1,0		RS-F	
4b	24,0	-0,21		SP	
1	19,3	-0,95		VT	

° calculation see p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

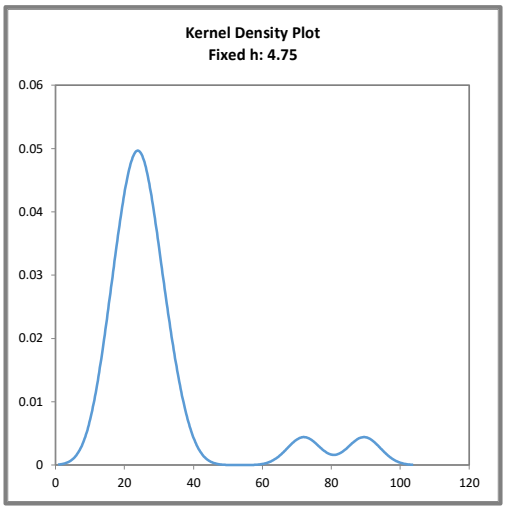


Abb. / Fig. 25:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows a symmetric distribution of results with two secondary peaks at 72 mg/kg and 90 mg/kg, due to single values of the method 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	19	11
Number of outliers	-	0
Mean	30,0	22,7
Median	24,8	24,0
Robust Mean (X_{pt})	**25,3**	**22,7**
Robust standard deviation (S*)	**7,21**	**4,70**
Target range:		
Target standard deviation σ_{pt}	**6,33**	**5,68**
lower limit of target range	**12,7**	**11,4**
upper limit of target range	**38,0**	**34,1**
Quotient S/σ_{pt}*	*1,1*	*0,83*
Standard uncertainty $U_{(Xpt)}$	*2,07*	*1,77*
Results in the target range	*17*	*11*
Percent in the target range	*89*	*100*

Methoden:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed nearly a symmetrical distribution of results with two high single values. The evaluation with robust statistics was not affected.

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

The robust means of the evaluations were 127% and 114% of the spiking level of gluten to the spiking level sample and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.58 "Recovery rates ELISA for gluten").

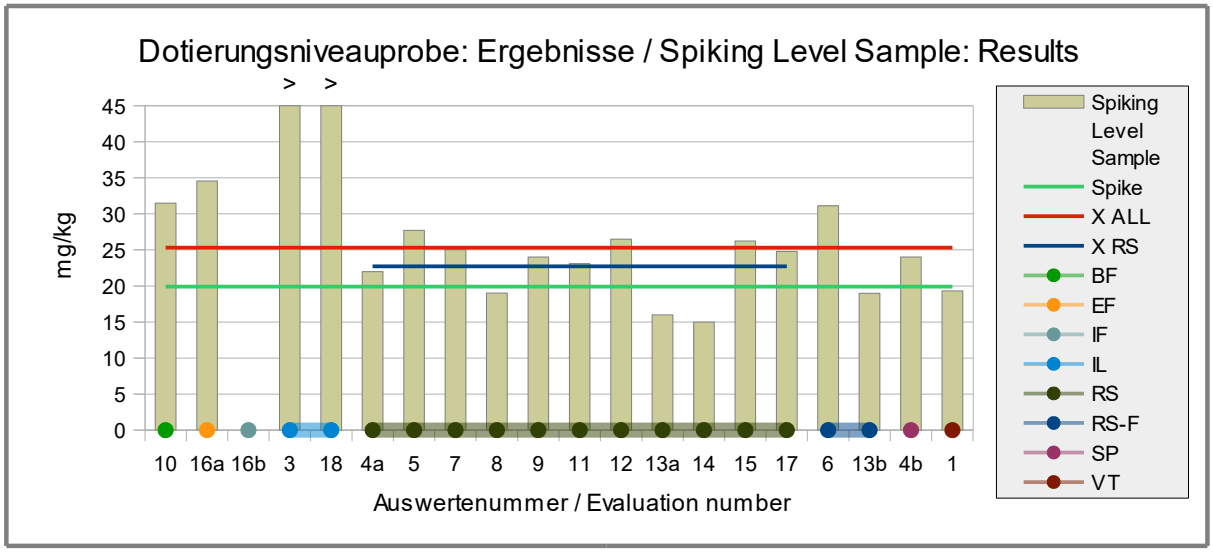


Abb./Fig. 26: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

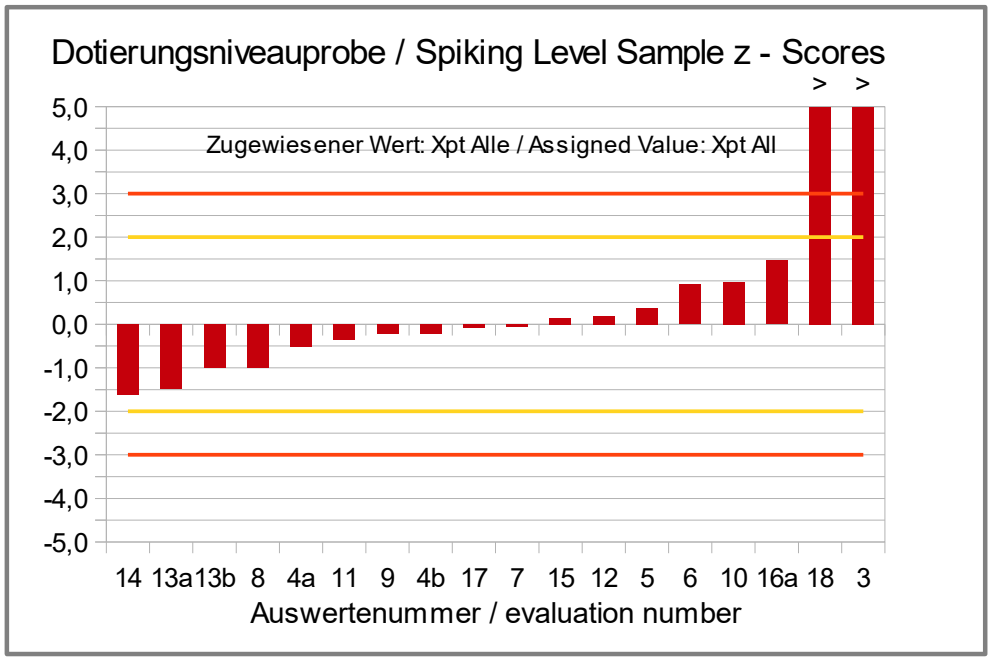


Abb./Fig. 27:
z-Scores ELISA Results Gluten
Assigned value robust mean of all results

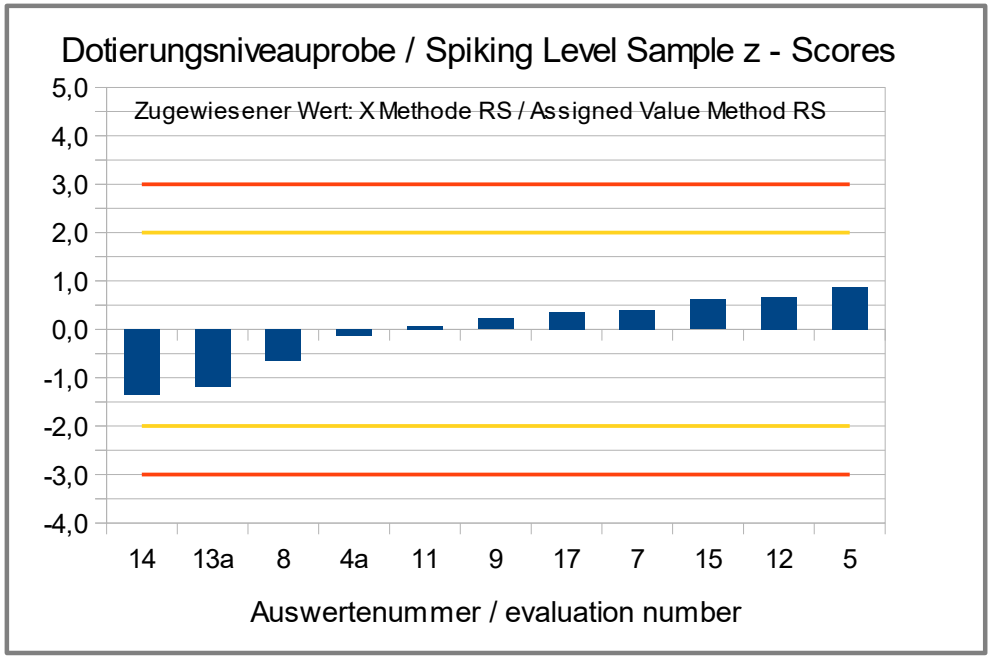


Abb./Fig. 28:
z-Scores ELISA Results Gluten Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
10	31,5	158	2,3	25,5	**115**	0,6	BF	
16a	34,6	174	3,0	38,8	175	3,0	EF	
16b							IF	
3	89,5	450	14	87,5	396	12	IL	
18	72,0	362	10	42,0	190	3,6	IL	Result converted °
4a	22,0	**111**	0,42	22,0	**100**	-0,02	RS	
5	27,7	**139**	1,6	28,9	**131**	1,2	RS	
7	25,0	**126**	1,0	15,0	**68**	-1,29	RS	
8	19,0	**96**	-0,17	21,4	**97**	-0,12	RS	
9	24,0	**121**	0,82	27,7	**125**	1,0	RS	
11	23,1	**116**	0,65	23,1	**104**	0,18	RS	
12	26,5	**133**	1,3	25,7	**116**	0,7	RS	
13a	16,0	**80**	-0,78	14,0	**63**	-1,5	RS	
14	15,0	**75**	-1,0	16,5	**75**	-1,01	RS	
15	26,2	**132**	1,3	14,6	**66**	-1,36	RS	
17	24,8	**125**	0,98	31,0	**140**	1,6	RS	
6	31,1	156	2,3	25,9	**117**	0,7	RS-F	
13b	19,0	**95**	-0,18	17,0	**77**	-0,92	RS-F	
4b	24,0	**121**	0,82	22,0	**100**	-0,02	SP	
1	19,3	**97**	-0,12	14,3	**65**	-1,4	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**14**	Number in RA	**16**
Percent in RA	**74**	Percent in RA	**84**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = ELISAFast, ifp
IF = ImmunoFast (Lateral Flow), ifp
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

74% (14) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A 84% (16) of the obtained recovery rates were within the recommended range.
The related z-scores are based on the target standard deviation of 25%.

4.2.2 PCR 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		
5	positive		negative	< 0,4	2/2 (100%)	SFA-ID	

	Sample A		Sample B	
Spiking	positive		negative	

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

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

Qualitative valuation of results: Spiking level sample

Evaluation number	Gluten	Gluten	Method	Remarks
	pos/neg	[mg/kg]		
5	positive		SFA-ID	

Spiking Level Sample	
Spiking	positive

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

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

4.3 Participant z-Scores: overview table

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

Evaluation number	ELISA β-Lacto-globulin: **Xpt** (div. methods)		ELISA β-Lacto-globulin: **Xpt** (method: RS-F)		ELISA Casein: **Xpt** (div. methods)		ELISA Casein: **Xpt** (method: RS-F)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	-	-	-	-	-	-	-	-
2	-1,9	-0,94	-	-	-	-	-	-
3	-2,7	-2,4	-	-	-2,6	-0,56	-	-
4 / 4a	-1,1	0,19	-	-	0,99	0,17	-	-
4b	-	-	-	-	-	-	-	-
5 /5a	-	-	-	-	-1,7	-1,7	-	-
5b	-	-	-	-	-1,1	-	-1,6	-
6	2,2	1,5	1,5	1,3	1,8	1,8	0,84	0,78
7	-0,17	2,9	-0,63	2,6	0,16	1,6	-0,54	0,66
8	-0,36	-1,8	-0,79	-1,9	0,40	0,16	-0,34	-0,56
9	0,62	-1,5	0,07	-1,7	0,10	1,8	-	-
10	-	-	-	-	-0,87	-1,4	-	-
11	-	-	-	-	-0,35	2,3	-	-
12	0,66	-0,13	0,11	-0,32	2,5	1,2	1,4	0,33
13 /13a	-	-	-	-	-	-2,3	-	-
13b	-	-	-	-	-	-	-	-
14	0,26	-0,03	-0,25	-0,22	1,0	-0,64	0,19	-1,2
15	1,0	0,53	0,41	0,31	-	-	-	-
16 /16a	1,2	2,0	-	-	-1,6	-2,3	-	-
16b	-	-	-	-	-	-	-	-
17	5,8	2,1	-	-	-1,6	-3,6	-	-
18	-2,8	-2,4	-	-	4,3	3,1	-	-

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

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

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

Evaluation number	ELISA Milk: **Xpt** (div. methods)		ELISA Gluten: **Xpt** (div. methods)		ELISA Gluten: **Xpt** (method: RS)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	-	-	-1,6	-0,95	-	-
2	-	-	-	-	-	-
3	-	-	11	10	-	-
4 / 4a	-	-	-0,34	-0,53	0,04	-0,13
4b	-	-	-0,34	-0,21	-	-
5 /5a	-	-	0,80	0,38	1,3	0,87
5b	-	-	-	-	-	-
6	0,10	0,16	0,31	0,91	-	-
7	-	-	-1,5	-0,05	-1,2	0,40
8	0,07	-0,59	-0,44	-0,99	-0,07	-0,65
9	-	-	0,60	-0,21	1,1	0,22
10	-	-	0,24	0,98	-	-
11	-	-	-0,16	-0,35	0,23	0,07
12	-	-	0,27	0,19	0,71	0,66
13 /13a	-	-	-1,7	-1,5	-1,4	-1,2
13b	-	-	-1,2	-1,0	-	-
14	-	-	-1,3	-1,6	-0,97	-1,4
15	0,52	0,00	-1,6	0,14	-1,3	0,62
16 /16a	-0,01	0,90	2,4	1,5	-	-
16b	-	-	-	-	-	-
17	-	-	1,2	-0,08	1,7	0,36
18	-3,4	-3,13	3,0	7,4	-	-

Methods: RS = Ridascreen®, R-Biopharm

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):

-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

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

Evaluation number	ELISA β-Lacto-globulin: **Xpt** (spiked level)		ELISA Casein: **Xpt** (spiked level)		ELISA Milch: **Xpt** (spiked level)		ELISA Gluten: **Xpt** (spiked level)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	-	-	-	-	-	-	-1,4	-0,12
2	-2,7	-2,3	-	-	-	-	-	-
3	-3,2	-3,1	-2,9	-0,25	-	-	12	14
4 / 4a	-2,2	-1,7	0,11	0,55	-	-	-0,02	0,42
4b	-	-	-	-	-	-	-0,02	0,82
5 /5a	-	-	-2,1	-1,5	-	-	1,2	1,6
5b	-	-	-1,6	-	-	-	-	-
6	-0,11	-0,95	0,80	2,3	9,6	7,2	0,69	2,3
7	-1,6	-0,19	-0,57	2,1	-	-	-1,3	1,0
8	-1,7	-2,8	-0,37	0,53	9,5	5,2	-0,12	-0,17
9	-1,1	-2,6	-0,62	2,3	-	-	1,0	0,82
10	-	-	-1,4	-1,2	-	-	0,62	2,3
11	-	-	-0,99	2,9	-	-	0,18	0,65
12	-1,1	-1,9	1,4	1,7	-	-	0,65	1,3
13 /13a	-	-	-	-2,1	-	-	-1,5	-0,78
13b	-	-	-	-	-	-	-0,92	-0,18
14	-1,3	-1,8	0,16	-0,34	-	-	-1,0	-0,98
15	-0,86	-1,5	-	-	11	6,8	-1,4	1,3
16 /16a	-0,72	-0,69	-2,0	-2,1	9,2	9,2	3,0	3,0
16b	-	-	-	-	-	-	-	-
17	2,2	-0,63	-2,0	-3,6	-	-	1,6	0,98
18	-3,2	-3,1	2,9	3,7	-2,2	-1,6	3,6	10

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):

-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)

-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)

-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: β-Lactoglobulin

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	5a		positive	>400	negative	< 0,01	positive	>400		0,01		beta-Lactoglobulin	AgraQuant ELISA β-Lactoglobulin COLAL1048, RomerLabs
EF	16a	25.03.20	positive	23,19	negative	0	positive	24,36	1,5	3		beta-Lactoglobulin	ELISAFast® β-Lactoglobulin, ifp
ES	13		positive	>1	negative	<0,1	positive	>1		0		beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
IF	16b	09.04.20	positive		negative		positive		0,2				ImmunoFast ® β-Lactoglobulin, ifp
IL	3	31.03.20	positive	5,7	negative	< 0,01	positive	6,5	0,01	0,01		beta-Lactoglobulin	Immunolab Beta-Lactoglobulin ELISA
MI-II	4	20.03.20	positive	13	negative	<0,031	positive	17	0,031	0,031		beta-Lactoglobulin	Morinaga Beta-lactoglobulin ELISA Kit II (M2112)
RS	2	03.04.20	positive	9,53	negative		positive	12,43	0,79	2,63	31	beta-Lactoglobulin	Ridascreen® β-Lactoglobulin R4901, R-Biopharm
RS	17	22/05	-	43,6	-	<	-	24,8		0		beta-Lactoglobulin	Ridascreen® β-Lactoglobulin R4901, R-Biopharm
RS-F	5b		positive	>4,5	negative	< 0,17	positive	>4,5		0,17		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	6	23.04.20	positive	27,5	negative	<LOD	positive	22,43	0,04	0,167		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	7	23.04.20	positive	17	negative	< 0,5	positive	28		0,5	50	beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	8	22.04.20	positive	16,17	negative		positive	8,76	0,04	0,167		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	9	17.04.	positive	20,5	negative		positive	10	0,5	1		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	12	April/May	positive	20,7	negative	< 0,167	positive	15,7	0,04	0		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	14	30.04.20	positive	18,9	negative	< 0,2	positive	16,1	0,2	1		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	15	21.04.20	positive	22,24	negative	<0,167	positive	18,39	0,04	0		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
SP	18	19.03.20	positive	5,4	negative	<0.01	positive	6,6	0.002	0.01		beta-Lactoglobulin	SensiSpec ELISA Beta-Lactoglobulin, Eurofins

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

Continuation ELISA β-Lactoglobulin:

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	5a			no	
EF	16a			yes	
ES	13				
IF	16b			yes	
IL	3				
MI-II	4	recognizes cow's milk-ß Lac	according to manufacturer's instructions	yes	
RS	2	Anti-BLG	washing buffer, 10 minutes, 50°C	No	
RS	17			no	we not found repeatability for sample A and sample C for the beta-lactoglobulin test
RS-F	5b			yes	
RS-F	6		Extraction solution: Extractor 2+Allergen extraction buffer containing Additive 1, time: approx. 30 min., temperature: 100 °C	yes	
RS-F	7			yes	
RS-F	8		according to test kit description	no	
RS-F	9	beta-Lactoglobulin			
RS-F	12	ß-lactoglobulin from cow's milk	Measurements sample A (mg/kg): 22,6; 19,4; 20,7; 20,2 Measurements spiking level sample (mg/kg): 18,2; 13,8; 16,5; 14,3		
RS-F	14			yes	
RS-F	15		according to the test kit instructions	no	
SP	18				

5.1.2 ELISA: Casein

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	5a		positive	8,4	negative	< 1	positive	11		1		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	9	19.03.	positive	14,8	negative		positive	27,7	0,2	0		Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	11		positive	13,17	negative	<0,2	positive	30,33	0,04	0		Casein	AgraQuant Casein COKAL 1200, RomerLabs
BF	10	21/4	positive	11,3	negative	bLOQ	positive	12,3	0,11	1		Casein	MonoTrace Milk (Casein) ELISA kit, BioFront Technologies
EF	16a	25.03.20	positive	8,69	negative	0	positive	8,33	0,2	1		Casein	ELISAFast® Casein, ifp
ES	13		positive	>10	negative	<1	positive	8,3		1		Casein	ELISA Systems Casein ESCASPRD-48
ES	17	20/04	-	8,8	-	<	-	1,9		0	31	Casein	ELISA Systems Casein ESCASPRD-48
IF	16b	09.04.20	positive		negative		positive		1				ImmunoFast ® Casein, ifp
IL	3	31.03.20	positive	4,9	negative	< 0,2	positive	16,5	0,2	0,2		Casein	Immunolab Casein ELISA
MI-II	4	20.03.20	positive	18	negative	<0,25	positive	20	0,25	0,25		Casein	Morinaga Casein ELISA Kit II (M2113)
RS-F	5b		positive	10,5	negative	< 0,5	positive	>13,5		0,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	6	23.04.20	positive	21	negative	<LOD	positive	27,7	0,71	2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	7	23.04.20	positive	15	negative	< 2,5	positive	27		2,5	32,2	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	8	23.04.20	positive	15,87	negative		positive	19,94	0,12	0,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	12	April/May	positive	23,6	negative	< 2,5	positive	25,1	0,71	3		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	14	20.05.20	positive	18,2	negative	< 0,71	positive	16,1	0,71	3		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
SP	18	20.03.20	positive	30	negative	< 0.1	positive	34	0.04	0.2		Casein	SensiSpec ELISA Casein, Eurofins

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	5a			no	
AQ	9	Casein			
AQ	11	Casein	extraction solution 15 min at 60°C, 1:10 dilution	no	
BF	10	Monoclonal antibody based assay	1:20 extraction for 10 minutes @ 60C	NO	
EF	16a			yes	
ES	13				
ES	17			yes	
IF	16b			yes	
IL	3				
MI-II	4	recognizes cow's milk casein	according to manufacturer's instructions	yes	
RS-F	5b			yes	
RS-F	6		Extraction solution: Extractor 2+Allergen extraction buffer containing Additive 1, time: approx. 30 min., temperature: 100 °C	yes	
RS-F	7			yes	
RS-F	8		according to test kit description (1 times allergen extraction buffer, 1 times extractor 2, buffer with AEP)	no	
RS-F	12	Cow's milk casein	Measurement sample A (mg/kg): 21,5; 25,2;22,8; 25,0 Measurement spiking level sample (mg/kg): 21,4; 23,9;29,9;		
RS-F	14			no	
SP	18				

5.1.3 ELISA: Milk

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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
ES	16a	12.05.20	positive	745,62	negative	0	positive	764,42	1	2		Skimmed milk powder	ELISAFast® Whole milk
IF	16b	06.05.20	positive		negative		positive		2				ImmunoFast ® Whole milk
RS-F	6	23.04.20	positive	252,48	negative	<LOD	positive	213,94	0,7	2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	8	28.04.20	positive	251,06	negative		positive	175,76	0,7	2,5		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
RS-F	15	21.04.20	positive	278,62	negative	<2,5	positive	205,87	0,7	3		Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
SP	18	24.03.20	positive	34	negative	< 0.2	positive	45	0.05	0.4		Casein+BLG	SensiSpec ELISA Milk, Eurofins

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
ES	16a			yes	The result is based on antibodies specific for alpha-lactalbumin
IF	16b			yes	
RS-F	6		Extraction solution: Extractor 2+Allergen extraction buffer containing Additive 1, time: approx. 30 min., temperature: 100 °C	yes	
RS-F	8		according to test kit description	yes	
RS-F	15		according to the test kit instructions	no	
SP	18				

5.1.4 ELISA: Gluten

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	10	16/4	positive	25,5	negative	bLOQ	positive	31,5	0,36	2		Gluten	MonoTrace Gluten ELISA kit, BioFront Technologies
EF	16a	06.04.20	positive	38,76	negative	0	positive	34,58	3	3		Gluten	ELISAFast® Gluten, ifp
IF	16b	09.04.20	positive		negative		positive		4				ImmunoFast ® Gluten, ifp
IL	3	31.03.20	positive	87,5	negative	< 4	positive	89,5	4	4		Gluten	Immunolab Gliadin/Gluten ELISA
IL	18	19.03.20	positive	21	negative	< 1	positive	36	0.3	2		Gliadin	Immunolab Gliadin/Gluten ELISA
RS	4a	23.03.20	positive	22	negative	<5	positive	22	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	5		positive	28,9	negative	< 3	positive	27,7		3		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	7	15.04.20	positive	15	negative	< 5	positive	25		5	17,6	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8	02.04.20	positive	21,41	negative		positive	19,03	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	9	19.03.	positive	27,7	negative		positive	24	5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	11		positive	23,08	negative	<5	positive	23,11	1	5		Gluten	Ridascreen Gliadin R7001, R-Biopharm
RS	12	April/May	positive	25,7	negative	< 5	positive	26,5	0,5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	13a		positive	14	negative	<5	positive	16		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	14	07.04.20	positive	16,5	negative	< 2,7	positive	15	2,7	5		Gliadin	Ridascreen® Gliadin R7001, R-Biopharm
RS	15	07.04.20	positive	14,59	negative	<5	positive	26,24	2,5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	17	20/04	-	31	-	<	-	24,8		5	31	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS-F	6	23.04.20	positive	25,9	negative	<LOD	positive	31,1	1	10		Gluten	Ridascreen® FAST Gliadin R7002, R-Biopharm
RS-F	13b		positive	17	negative	<2,5	positive	19		3		Gluten	Ridascreen®Fast Gliadin Sensitive R7051, R-Biopharm
SP	4b	19.03.20	positive	22	negative	<3,12	positive	24	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins
VT	1	02.04.20	14,3		0		19,3					Gluten	Veratox Gliadin R5, Neogen

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Gluten:

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BF	10	Monoclonal antibody based assay	1:40 extraction ratio for 1 hour @ 60c	NO	
EF	16a			yes	
IF	16b			yes	
IL	3				
IL	18				
RS	4a	Mendez's R5 antibody recognizes prolamins (gliadins) from wheat, rye and barley	according to manufacturer's instructions	yes	
RS	5			yes	
RS	7	R5		yes	
RS	8		according to test kit description	yes	
RS	9	Gliadins (R5 antibody)			
RS	11	Gliadin	Extraction with cocktail solution and ethanol, 40 min at 50°C, 1:500 dilution	no	
RS	12	Monoclonal antibody R5	Measurements sample A (mg/kg): 27,7; 21,1; 28,2 Measurements spiking level sample (mg/kg): 29; 26,1; 24,4		
RS	13a				
RS	14			yes	
RS	15	R5	according to the test kit instructions	no	
RS	17			yes	
RS-F	6	R5	Extraction solution: Coctail (patented), time: approx. 2 hours, temperature: 50 °C	yes	
RS-F	13b				
SP	4b	Mendez's R5 antibody recognizes prolamins (gliadins) from wheat, rye and barley	according to manufacturer's instructions	yes	
VT	1		40 min / 50°C	no	

5.1.5 PCR: Milk

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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
div	17	21/04	positive		negative		positive		0,01			DNA-Cow	Internal Method 207 Rev. 8

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

Meth. Abbr.	Evaluation no.	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	
div	17		Extraction> Nucleo Spin Food, Real Time PCRQuantStudio5. 7500 Fast and CFX-96 deep well	yes	

5.1.6 PCR: Gluten

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level 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	5		positive		negative	< 0,4	positive		0,4			Gluten	Sure Food Allergen ID, R-Biopharm / Congen

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

Meth. Abbr.	Evaluation no.	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	5			no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptA03 2020 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	33,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	96	38,2
2	4,97	100	40,2
3	5,01	98	39,1
4	5,01	88	35,1
5	5,02	88	35,1
6	5,00	105	42,0
7	5,04	90	35,7
8	5,03	103	41,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	96,0	Particles
Standard deviation	6,86	Particles
χ^2 (CHI-Quadrat)	3,43	
Probability	**84**	%
Recovery rate	115	%

Normal distribution		
Number of samples	8	
Mean	38,3	mg/kg
Standard deviation	2,74	mg/kg
rel. Standard deviaton	7,14	%
Horwitz standard deviation	9,24	%
HorRat-value	**0,77**	
Recovery rate	115	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL03 - 2020***
PT name	***Allergens III: β-Lactoglobulin, Casein and Gluten in Infant Food (pap, heated) with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *4-grain pap "gluten-free" and "milk-free" (prepared, heated to 50 °C) / ingredients: water, whole rice flour 32%, maize flour 30%, whole millet flour 23%, whole buckwheat flour 15%, 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 + Spiking Level Sample: cooled 2 - 10°C (PT period)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: β-Lactoglobulin, Casein and Gluten (Gluten-containing Cereals) Samples A + B: < 500 mg/kg Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is recommended to homogenize the entire sample amount.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest April 24th 2020.***
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	*Alexandra Scharf M.Sc.*

** 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
		USA
		Germany
		CANADA
		ITALY
		Germany
		SWITZERLAND
		Germany
		AUSTRIA
		Germany
		Germany
		SPAIN
		Germany
		ITALY
		Germany
		Germany
		HUNGARY
		NETHERLANDS
		SPAIN
		Germany

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked im-

munoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 16.01-9 Untersuchung von Lebensmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016) [Foodstuffs, determination of soya (Glycine max) in cereal flour by real-time PCR]
33. ASU §64 LFGB L 08.00-59 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
34. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2017) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]
35. ASU §64 LFGB L 08.00-66 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Weizen (Triticum L.) und Roggen (Secale cereale) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of wheat (Triticum L.) and rye (Secale cereale) in boiled sausages by real-time PCR]
36. Allergen Data Collection - Update (2002): Cow's Milk (Bos domesticus), Besler M., Eigenmann P., Schwartz R., Internet Symposium on Food Allergens 4(1): 19-106, http://www.food-allergens.de
37. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Informationen für Verbraucher über das Nichtvorhandensein oder das reduzierte Vorhandensein von Gluten in Lebensmitteln / on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food
38. Köhler & Andersen (2014) Analyse von Glutengehalten in Getreide und getreidehaltigen Produkten, Tabellenwerk zum Nährstoffgehalt von Lebensmitteln 3.1.5.1, Deutsche Forschungsanstalt für Lebensmittelchemie Leibniz Institut Jahresbericht 2014 [Analysis of gluten contents in cereals and cereal products, nutrient tables of foods]