Proficiency Tests
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Evaluation Report
proficiency test

DLA 03/2018

Allergens III:

β-Lactoglobulin, Casein and Gluten

in Infant Food

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2nd Correction 25/07/2018:
There was a mistake in the table "Recovery Rates ELISA for Gluten" on page 50:
The recovery rates for sample A and the spiking level sample were calculated with mixed up reference values for gluten of 37,7 mg/kg and 36,1 mg/kg, respectively.
This has been corrected.

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

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 25 July 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material was a mixture of common in commerce infant food products "cereal pap" for children from up to 4 and 6 month (labeled as milk- 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.
Afterwards the **spiked sample A** was produced as follows:
The spiking materials (premix) containing the allergenic ingredients skimmed milk powder and wheat flour were sieved by means of a centrifugal mill (mesh 250 µm and 500 µm, respectively), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 3 additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh 500 µm) and homogenization.

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

Table 1: Composition of the DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Organic-Cereal-Pap, infant pap after 4th and 6th month Ingredients: Millet whole flour (68%), brown rice flour (25%), corn whole flour (5,4%), buckwheat flour (1,6%), thiamine Nutrients per 100g: Fat 3,6 g, carbohydrates 77 g, protein 11 g	99,7 g/100 g	100 g/100g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,7 g/100 g
Milk: Skimmed milk powder mixture (9 products from Europe, USA) - as skimmed milk powder* - thereof 33,0% total protein** - thereof casein*** - thereof β-lactoglobulin***	 215 mg/kg 71,0 mg/kg 56,8 mg/kg 7,1 mg/kg	-	 236 mg/kg 77,9 mg/kg 62,3 mg/kg 7,8 mg/kg
Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) - as wheat flour* - thereof 10,1% total protein** - thereof gluten***	 433 mg/kg 43,7 mg/kg 37,7 mg/kg	-	 415 mg/kg 41,9 mg/kg 36,1 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	<0,3 g/100 g	-	<0,3 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw materials (total nitrogen according to Kjeldahl with F=6,38 for milk protein and F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 80% casein and 10% β-latcoglobulin in total milk protein [36]; approx. 8,7% gluten in wheat flour [37, 38, 39])

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT sample A showed a probability of 92% and 80% for the spiking level sample, respectively. 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 HorRat values of 0,76 and 0,84, respectively. 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 gluten (Immunolab, Veratox, AgraQuant and AgraQuant G12) and milk/casein (Immunolab, Veratox and AgraQuant) (see pages 7-9). 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].

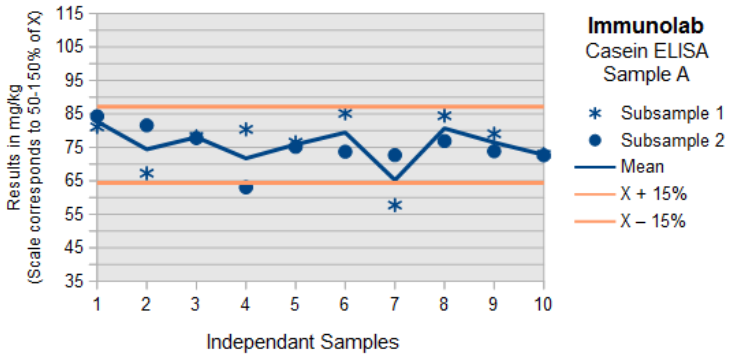
ELISA-Tests: Homogenität Milch / Homogeneity Milk

Immunolab Casein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Casein 75,7 ± 3,7 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	81,1	84,3	82,7
2	67,2	81,6	74,4
3	78,3	77,8	78,0
4	80,3	63,0	71,7
5	76,5	75,1	75,8
6	85,1	73,7	79,4
7	57,7	72,7	65,2
8	84,4	76,9	80,6
9	79,0	73,8	76,4
10	72,9	72,6	72,8

General average X	75,7	
SD of sample means Sx	5,05	6,7%
SD w ithin-samples Sw	4,89	6,5%
SD betw een-samples Ss	3,68	4,9%

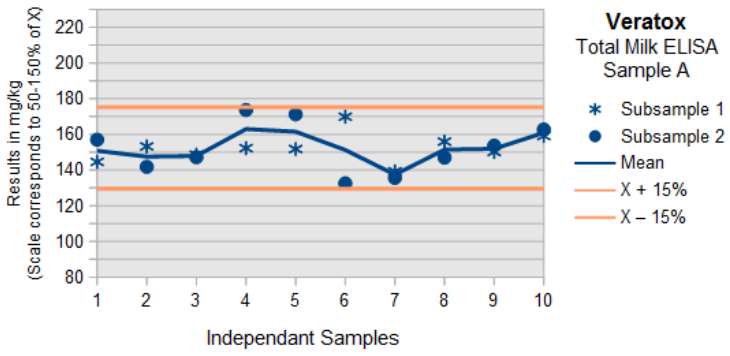


Neogen Veratox Total Milk

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Skimmed Milk Powder 152 ± 5,2 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	145	157	151
2	153	142	148
3	149	147	148
4	152	174	163
5	152	171	162
6	170	133	151
7	139	136	137
8	156	147	151
9	150	154	152
10	159	163	161

General average X	152	
SD of sample means Sx	7,74	5,1%
SD w ithin-samples Sw	8,11	5,3%
SD betw een-samples Ss	5,19	3,4%

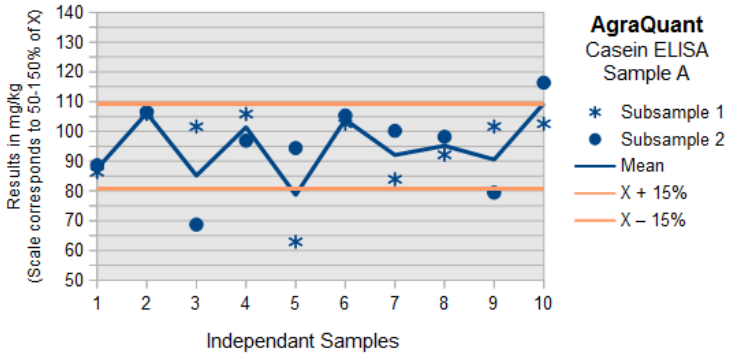


Romerlabs AgraQuant Casein

Sample weights: 0,50 g (0,45 – 0,55 g)
Number of replicates: 2
Overall result: Casein 95,0 ± 7,8 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	86,3	88,6	87,5
2	106	106	106
3	102	68,7	85,2
4	106	96,9	101
5	62,9	94,4	78,7
6	103	105	104
7	83,9	100	92,1
8	92,2	98,2	95,2
9	102	79	90,5
10	103	116	109

General average X	95,0	
SD of sample means Sx	10,03	10,6%
SD w ithin-samples Sw	8,88	9,3%
SD betw een-samples Ss	7,82	8,2%



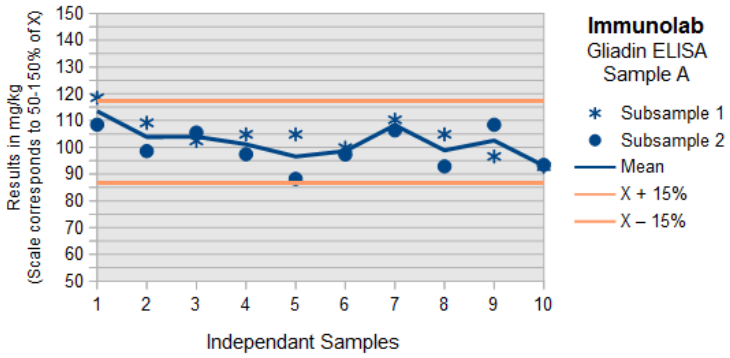
ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 102 ± 4,9 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	119	108	113
2	109	98,6	104
3	102	106	104
4	105	97,4	101
5	105	88,2	96,5
6	100	97,3	98,6
7	110	106	108
8	105	92,9	98,9
9	96,6	108	103
10	92,8	93,4	93,1

General average X	102	
SD of sample means Sx	5,87	5,8%
SD w ithin-samples Sw	4,64	4,5%
SD betw een-samples Ss	4,87	4,8%

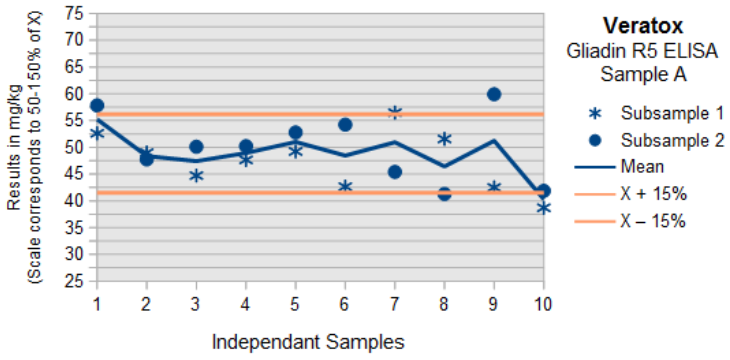


Neogen Veratox ELISA Gliadin R5

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Gluten 48,8 ± 2,4 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	52,6	57,8	55,2
2	49,0	47,7	48,4
3	44,7	50,1	47,4
4	47,6	50,2	48,9
5	49,2	52,8	51,0
6	42,7	54,2	48,4
7	56,5	45,4	50,9
8	51,6	41,3	46,4
9	42,5	59,9	51,2
10	38,7	41,9	40,3

General average X	48,8	
SD of sample means Sx	3,90	8,0%
SD w ithin-samples Sw	4,33	8,9%
SD betw een-samples Ss	2,41	4,9%

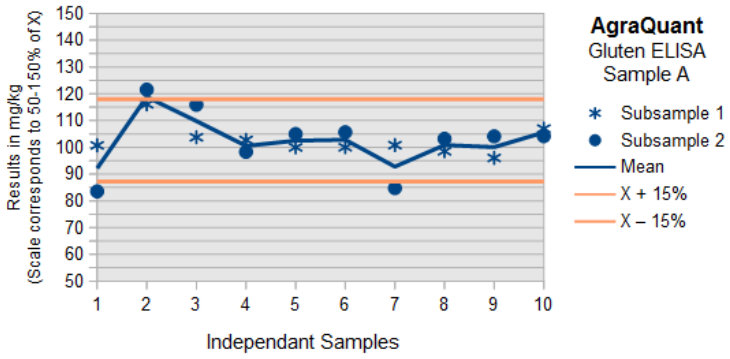


Romerlabs AgraQuant Gluten

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 103 ± 7,0 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	101	83,5	92,1
2	116	122	119
3	104	116	110
4	103	98,3	101
5	100	105	102
6	100	106	103
7	101	84,7	92,8
8	98,5	103	101
9	96,0	104	100
10	107	104	106

General average X	103	
SD of sample means Sx	7,77	7,6%
SD w ithin-samples Sw	4,75	4,6%
SD betw een-samples Ss	7,01	6,8%

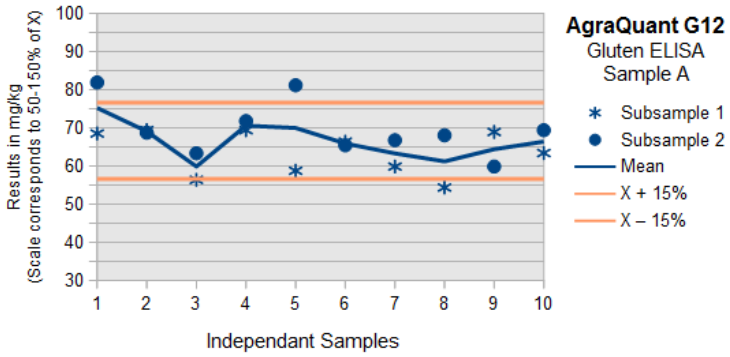


Romerlabs AgraQuant Gluten G12

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 66,6 ± 2,9 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	68,5	81,9	75,2
2	69,4	68,7	69,1
3	56,4	63,3	59,8
4	69,4	71,8	70,6
5	58,7	81,1	69,9
6	66,4	65,4	65,9
7	59,8	66,7	63,3
8	54,3	68,0	61,2
9	68,9	59,8	64,4
10	63,4	69,4	66,4

General average X	66,6	
SD of sample means Sx	4,69	7,1%
SD within-samples Sw	5,22	7,8%
SD between-samples Ss	2,90	4,4%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,28 (24,5°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A, B and the spiking level sample were sent to every participating laboratory in the 11th week of 2018. The testing method was optional. The tests should be finished at April 27th 2018.

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/or Gluten in the range of mg/kg in the matrix of infant food (pap powder with sorghum, rice, maize and buckwheat). One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing.

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

2.3 Submission of results

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

All 18 participants submitted their results in time.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 11 – 32% for the ELISA methods and 18 – 38% 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} [32-36]

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

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

too low with respect to the standard uncertainty of the assigned value.

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

3.9 Figures

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

3.10 Recovery rates: Spiking

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

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done separately for ELISA and PCR-techniques. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

The following result sections are structured equally for the allergenic components. First all results of ELISA or PCR methods for a certain parameter are reported for samples A and B (qualitative / possibly quantitative) and afterwards for the spiking level sample (quantitative). The recovery rates of results for the spiking level sample and the spiked sample A or B are reported then.

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

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

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

Casein-specific ELISA results given as **skimmed milk powder** were converted to **casein**. For this the information supplied in the manufacturer's test kit instructions for the content of casein in skimmed milk powder were taken (ELISA-Systems Test-Kit Manual: 25,6%, Neogen Allergen Handbook: 28,8%).

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

All gluten ELISA results were submitted as gluten, therefore no conversion was necessary.

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

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

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

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

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

Characteristics	**All Results** *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* $\sigma_{pt'}$		
lower limit of target range (X_{pt} - $2\sigma_{pt}$) or (X_{pt} - $2\sigma_{pt'}$)°		
upper limit of target range (X_{pt} + $2\sigma_{pt'}$) or (X_{pt} + $2\sigma_{pt'}$)°		
Quotient S*/σ_{pt} *or* S*/$\sigma_{pt'}$		
Standard uncertainty $U_{(Xpt)}$		
Number of results in target range		
Percent in target range		

° Target range is calculated with z-score or z'-score

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

4.1 Proficiency Test β-Lactoglobulin

4.1.1 ELISA Results: β-Lactoglobulin

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
9	positive	>0,4	positive	0,01	1/2 (50%)	AQ	
6	positive	14,8	negative		2/2 (100%)	BK	
1a	positive	8,00	negative	N/A	2/2 (100%)	ES	
7	positive	19,6	negative	<0,1	2/2 (100%)	ES	
12	positive	>1	negative	<0,1	2/2 (100%)	ES	
1b	positive	3,66	negative	N/A	2/2 (100%)	MI	result converted °
15	positive	4,20	negative	<0,031	2/2 (100%)	MI	
3	positive	1,80	negative		2/2 (100%)	NL	
4	positive	3,28	negative	<2,63	2/2 (100%)	RS	
16	negative	<5	negative	<5	1/2 (50%)	RS	no positive sample detected
5	positive	7,10	negative	<0,167	2/2 (100%)	RS-F	
10	positive	1,66	negative		2/2 (100%)	RS-F	
13	positive	5,00	negative	< 0,5	2/2 (100%)	RS-F	
14	positive	2,24	negative	<0,5	2/2 (100%)	RS-F	
2	positive	>0,5	positive	0,08	1/2 (50%)	ZL	

° calculation p. 20

	Sample A		Sample B	
Number positive	14		2	
Number negative	1		13	
Percent positive	93		13	
Percent negative	7		87	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
ZL = Proteon ELISA, Zeulab

Comments:

The consensus values are in agreement with the spiking of sample A. One negative result for sample A was obtained with a LOD above the median of the other positive results. For sample B there were two positive results, the values were <0,1 mg/kg each.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	β-Lacto-globulin	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
9	>0,4		AQ	
6	14,8	5,6	BK	
1a	8,00	2,0	ES	
7	19,6	8,1	ES	
12	>1		ES	
1b	3,66	-0,28	MI	result converted °
15	4,20	0,00	MI	
3	1,80	-1,3	NL	
4	3,28	-0,48	RS	
16	<5		RS	
5	7,10	1,5	RS-F	
10	1,66	-1,3	RS-F	
13	5,00	0,42	RS-F	
14	2,24	-1,0	RS-F	
2	>0,5		ZL	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
ZL = Proteon ELISA, Zeulab

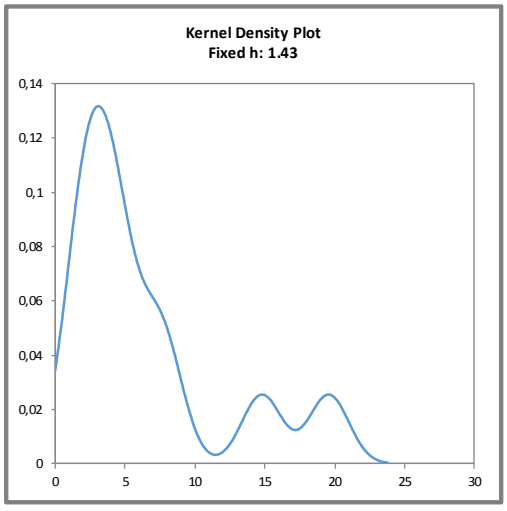


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 slight shoulder and two side-peak at approx. 15 mg/kg (method BK) and approx. 20 mg/kg (method ES).

Characteristics: Quantitative evaluation ELISA β-Lactoglobulin

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	11
Number of outliers	-
Mean	6,49
Median (X_{pt})	**4,20**
Robust Mean	5,51
Robust standard deviation (S*)	**4,22**
Target range:	
Target standard deviation $\sigma_{pt'}$	**1,91**
lower limit of target range	**0,39**
upper limit of target range	**8,01**
Quotient S/$\sigma_{pt'}$*	*2,2*
Standard uncertainty $U_{(Xpt)}$	*1,59*
Results in the target range	*9*
Percent in the target range	*82*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results without clear method-dependent differences.

The median was taken as the assigned value (see 3.1).

The results showed an increased variability, thus evaluation was done by z'-score considering the standard uncertainty (see 3.6 and 3.8).
The quotient S/$\sigma_{pt'}$ was 2,2. The robust standard deviation was increased and above the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods, because there were only a few results for some methods.*

The assigned value Xpt (median) of the evaluation of all results was 59% of the spiking level of "β-lactoglobulin" to sample A and thus above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of β-Lactoglobulin" p.29).

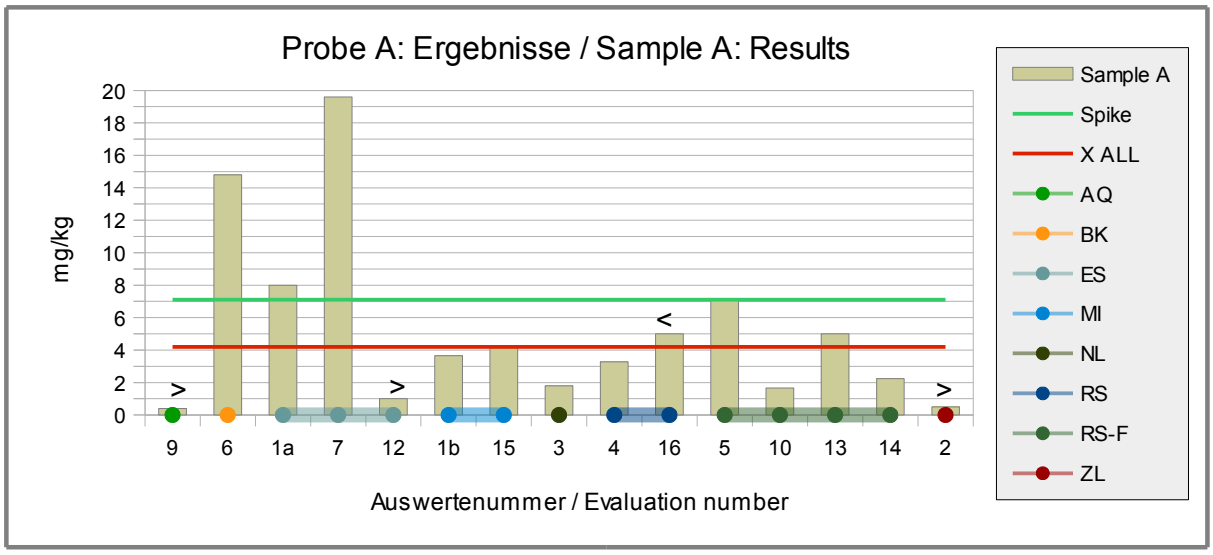


Abb./Fig. 2: ELISA Results β-Lactoglobulin
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

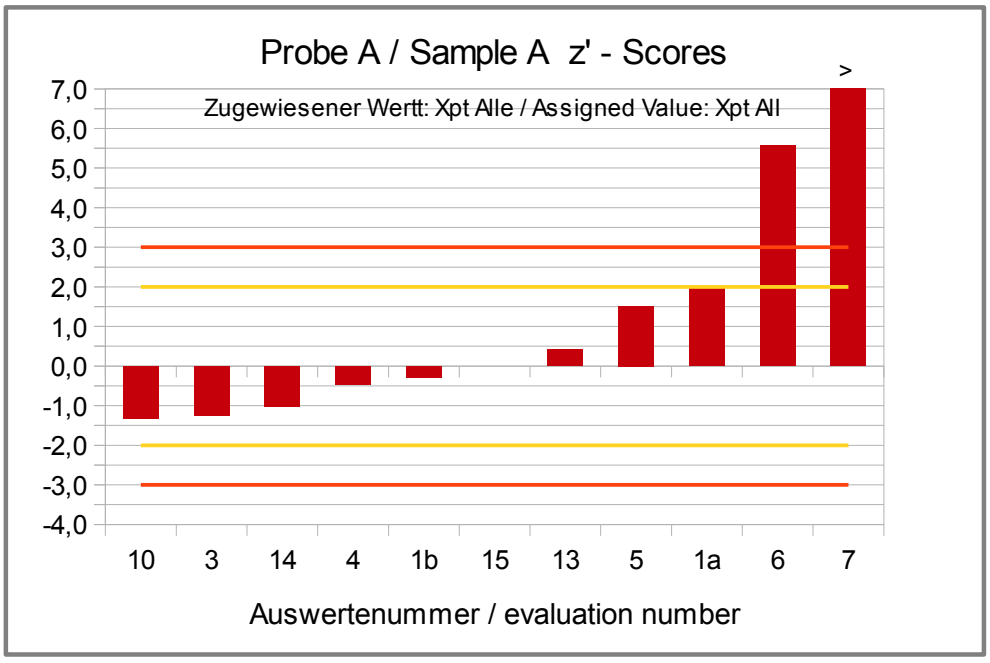


Abb./Fig. 3:
z'-Scores (ELISA Results β-Lactoglobulin) Assigned value median of all results

Quantitative evaluation of ELISA results: Spiking level sample

Evaluation number	β-Lacto-globulin	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
9	>0,4		AQ	
6	18,0	5,6	BK	
1a	9,30	1,9	ES	
7	21,5	7,1	ES	
12	>1		ES	
1b	4,83	-0,06	MI	result converted °
15	4,70	-0,11	MI	
3	1,90	-1,3	NL	
4	3,70	-0,55	RS	
16	<5		RS	no positive sample detected
5	5,68	0,31	RS-F	
10	2,95	-0,87	RS-F	
13	5,10	0,06	RS-F	
14			RS-F	
2	>0,5		ZL	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
ZL = Proteon ELISA, Zeulab

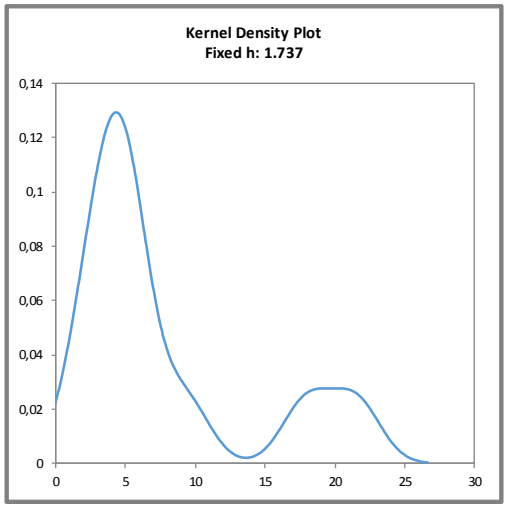


Abb. / Fig. 4:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 and two side-peak at approx. 18 mg/kg (method BK) and approx. 21,5 mg/kg (method ES).

Characteristics: Quantitative evaluation β-Lactoglobulin

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	10
Number of outliers	-
Mean	7,77
Median (X_{pt})	**4,97**
Robust Mean	6,62
Robust standard deviation (S*)	**4,95**
Target range:	
Target standard deviation $\sigma_{pt'}$	**2,32**
lower limit of target range	**0,334**
upper limit of target range	**9,60**
Quotient S/$\sigma_{pt'}$*	*2,1*
Standard uncertainty $U_{(Xpt)}$	*1,95*
Results in the target range	*8*
Percent in the target range	*80*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results without clear method-dependent differences.

The median was taken as the assigned value (see 3.1).

The results showed an increased variability, thus evaluation was done by z'-score considering the standard uncertainty (see 3.6 and 3.8).
The quotient S/$\sigma_{pt'}$ was 2,1. The robust standard deviation was increased and above the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is limited for the evaluation across the methods, because there were only a few results for some methods.*

The assigned value Xpt (median) of the evaluation of all results was 64% of the spiking level of "β-lactoglobulin" to the spiking level sample and thus above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of β-Lactoglobulin" p.29).

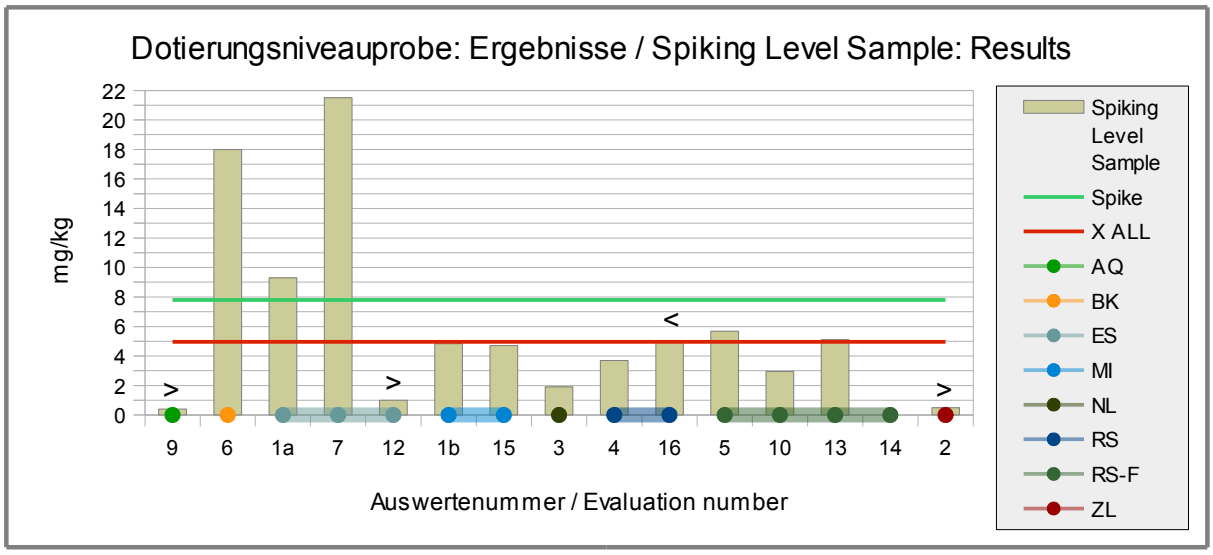


Abb./Fig. 5: ELISA Results β-Lactoglobulin
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

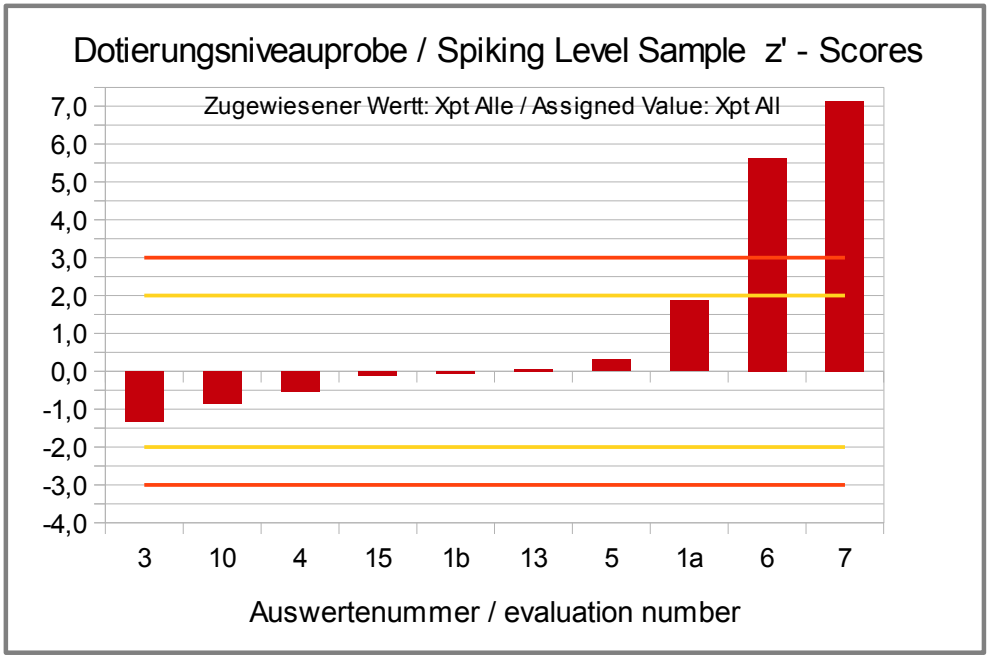


Abb./Fig. 6:
z'-Scores (ELISA Results β-Lactoglobulin)
Assigned value median of all results

Recovery Rates 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]	[%]	[mg/kg]	[%]		
9	>0,4		>0,4		AQ	
6	18,0	231	14,8	208	BK	
1a	9,30	**119**	8,00	**113**	ES	
7	21,5	276	19,6	276	ES	
12	>1		>1		ES	
1b	4,83	**62**	3,66	**52**	MI	result converted °
15	4,70	**60**	4,20	**59**	MI	
3	1,90	24	1,80	25	NL	
4	3,70	47	3,28	46	RS	
16	<5		<5		RS	
5	5,68	**73**	7,10	**100**	RS-F	
10	2,95	38	1,66	23	RS-F	
13	5,10	**65**	5,00	**70**	RS-F	
14			2,24	32	RS-F	
2	>0,5		>0,5		ZL	

° calculation p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**5**	Number in RA	**5**
Percent in RA	**50**	Percent in RA	**45**

* Recovery rate 100% relative size: β-Lactoglobulin, see p. 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
MI = Morinaga Institute ELISA Kit II
NL = nutriLinia® Allergen-ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
ZL = Proteon ELISA, Zeulab

Comments:

For the spiking level sample 50% (5) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 45% (5) of the participants obtained a recovery rate within the range of acceptance.

4.2 Proficiency test: Casein / Milk Protein

4.2.1 ELISA Results: Casein

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
6	positive	61,0	negative		2/2 (100%)	AQ	
12	positive	>2,6	negative	<0,26	2/2 (100%)	ES	result converted °
1a	positive	33,3	negative	N/A	2/2 (100%)	ES	result converted °
9	positive	>6,0	negative	0	2/2 (100%)	IL	
18	positive	87,0	negative	< 0.01	2/2 (100%)	IL	
1b	positive	45,2	negative	N/A	2/2 (100%)	MI	result converted °
3	positive	59,5	negative		2/2 (100%)	NL	
5	positive	38,4	negative	<2.5	2/2 (100%)	RS-F	
10	positive	114	negative		2/2 (100%)	RS-F	
13	positive	75,0	negative	< 0,5	2/2 (100%)	RS-F	
14	positive	>13,5	negative	<0,5	2/2 (100%)	RS-F	
7	positive	190	negative	<2,5	2/2 (100%)	VT	
15	positive	56,0	negative	<0,25	2/2 (100%)	div	

° calculation p. 20

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

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

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

Quantitative valuation of ELISA results: Sample A

Evaluation number	Casein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
6	61,0	-0,04	AQ	
12	>2,6		ES	result converted °
1a	33,3	-1,8	ES	result converted °
9	>6,0		IL	
18	87,0	1,6	IL	
1b	45,2	-1,1	MI	result converted °
3	59,5	-0,14	NL	
5	38,4	-1,5	RS-F	
10	114	3,4	RS-F	
13	75,0	0,87	RS-F	
14	>13,5		RS-F	
7	190	8,3	VT	outlier excluded
15	56,0	-0,36	div	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

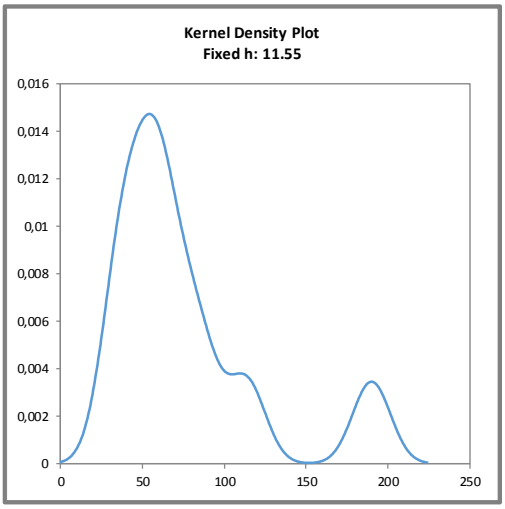


Abb. / Fig. 7:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 114 mg/kg (method RS-F) and a side peak at 190 mg/kg (method VT), which is due to an outlier (eventually submitted as casein by mistake, because according to test kit instructions the results are calibrated with skimmed milk powder).

Characteristics: Quantitative evaluation ELISA Casein

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results°	9
Number of outliers	1
Mean	63,2
Median	59,5
Robust Mean (X_{pt})	**61,6**
Robust standard deviation (S*)	**24,9**
Target range:	
Target standard deviation σ_{pt}	**15,4**
lower limit of target range	**30,8**
upper limit of target range	**92,4**
Quotient S/σ_{pt}*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*10,4*
Results in the target range	*8*
Percent in the target range	*89*

° *without result no. 7 (as outlier excluded)*

Comments to the statistical characteristics and assigned values:

The kernel density plot showed nearly a symmetrical distribution with one side peak. One outlier was excluded from statistical evaluation.

The evaluation of results of all methods showed a normal variability of results. The quotient S/σ_{pt} was below 2,0. The robust standard deviation is in the range of established values for the 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 assigned value X_{pt} (robust mean) of the evaluation of all results was 108% of the spiking level of casein to sample A and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for casein").

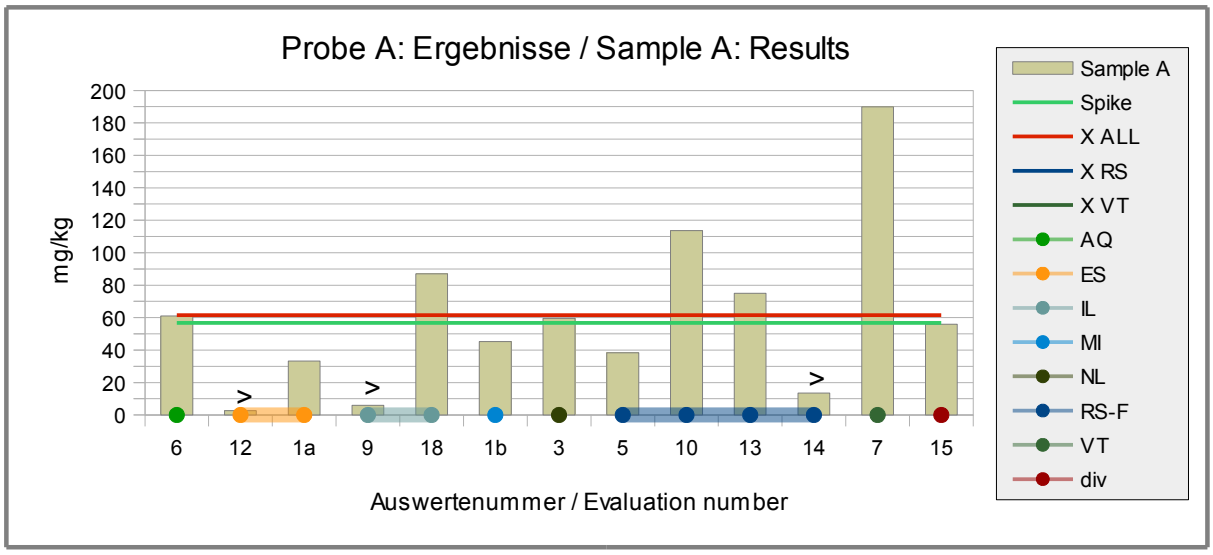


Abb./Fig. 8: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

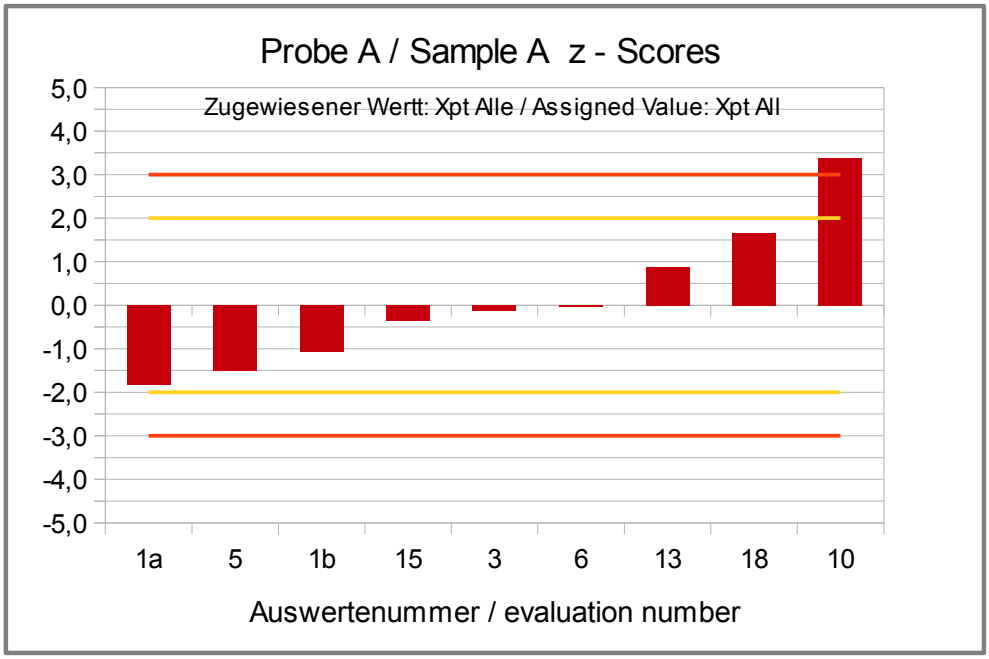


Abb./Fig. 9:
z-Scores (ELISA Results Casein) Assigned value robust mean of all results

Quantitative valuation of ELISA results: Spiking level sample

Evaluation number	Casein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
6	63,3	-0,21	AQ	
12	>2,6		ES	result converted °
1a	19,4	-2,8	ES	result converted °
9	>6,0		IL	
18	75,0	0,50	IL	
1b	85,4	1,1	MI	result converted °
3	60,5	-0,37	NL	
5	35,1	-1,9	RS-F	
10	87,3	1,2	RS-F	
13	103	2,2	RS-F	
14			RS-F	
7	207	8,4	VT	outlier excluded
15	67,0	0,02	div	

° calculation p. 20

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

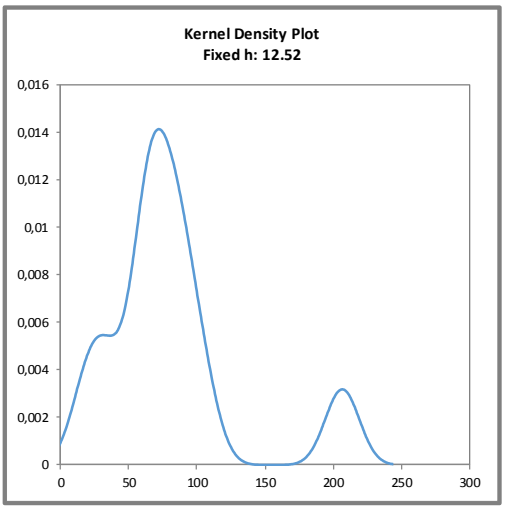


Abb. / Fig. 10:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 20 mg/kg (method ES) and a side peak at 207 mg/kg (method VT), which is due to an outlier (eventually submitted as casein by mistake, because according to test kit instructions the results are calibrated with skimmed milk powder).

Characteristics: Quantitative evaluation ELISA Casein

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results°	9
Number of outliers	1
Mean	66,2
Median	67,0
Robust Mean (X_{pt})	**66,7**
Robust standard deviation (S*)	**28,4**
Target range:	
Target standard deviation σ_{pt}	**16,7**
lower limit of target range	**33,4**
upper limit of target range	**100**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*11,8*
Results in the target range	*7*
Percent in the target range	*78*

° *without result no. 7 (as outlier excluded)*

Comments to the statistical characteristics and assigned values:

The kernel density plot showed nearly a symmetrical distribution with one side peak. One outlier was excluded from statistical evaluation.

The evaluation of results of all methods showed a normal variability of results. The quotient S/σ_{pt} was below 2,0. The robust standard deviation is in the range of established values for the 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 assigned value Xpt (robust mean) of the evaluation of all results was 107% of the spiking level of casein to the spiking level sample and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for casein").

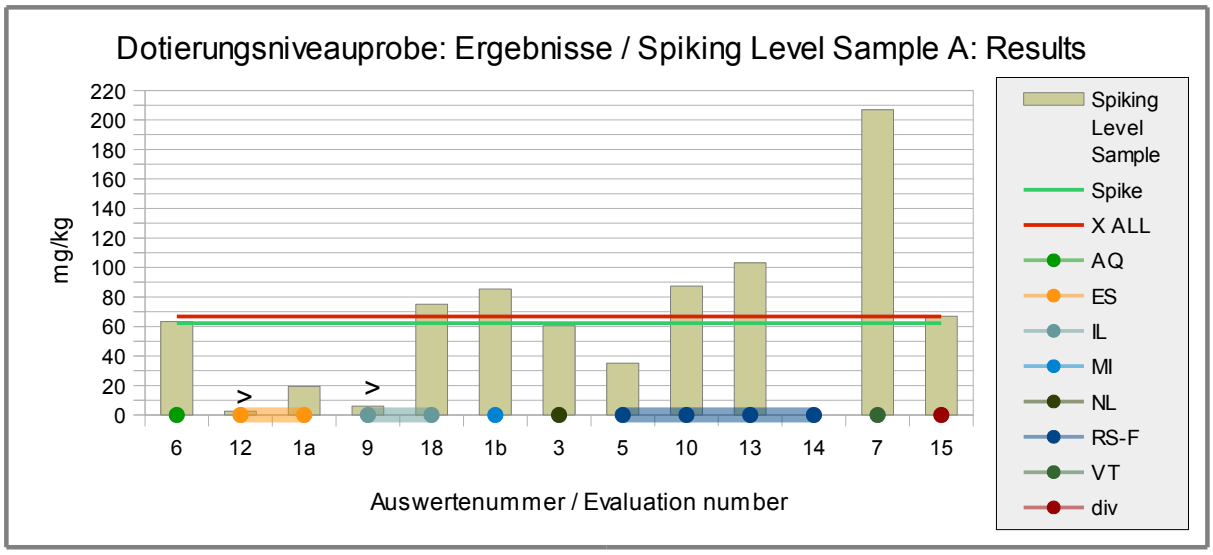


Abb./Fig. 11: ELISA Results Casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

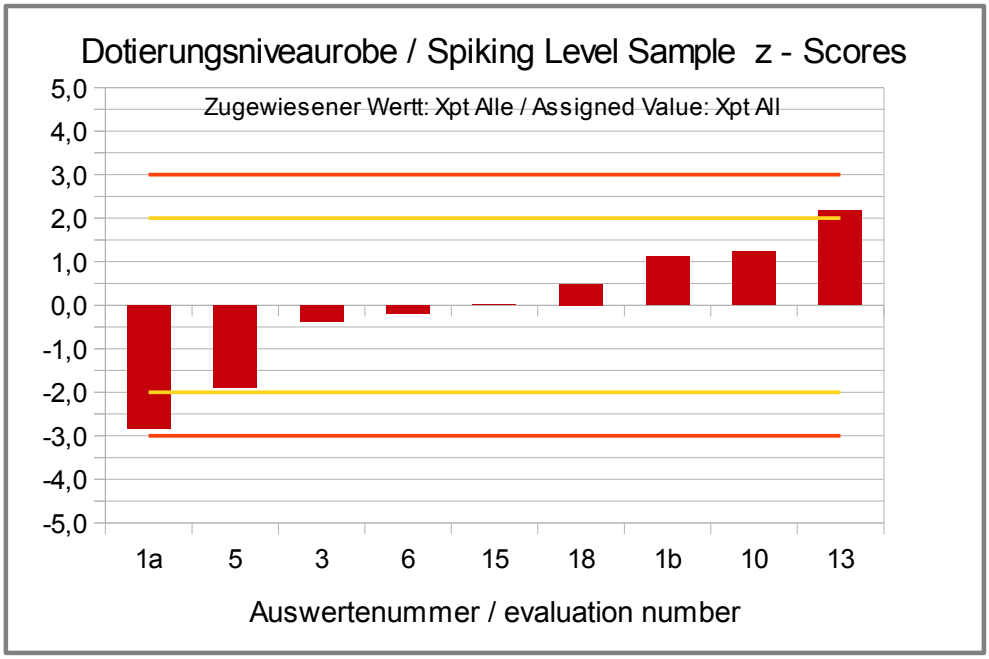


Abb./Fig. 12:
z-Scores (ELISA Results Casein) Assigned value robust mean of all results

Recovery Rates for Casein:
Spiking level sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6	63,3	**102**	61,0	**107**	AQ	
12	>2,6		>2,6		ES	result converted °
1a	19,4	31	33,3	**59**	ES	result converted °
9	>6,0		>6,0		IL	
18	75,0	**120**	87,0	**153**	IL	
1b	85,4	**137**	45,2	**80**	MI	result converted °
3	60,5	**97**	59,5	**105**	NL	
5	35,1	**56**	38,4	**68**	RS-F	
10	87,3	**140**	114	200	RS-F	
13	103	165	75,0	**132**	RS-F	
14			>13,5		RS-F	
7	207	332	190	334	VT	
15	67,0	**108**	56,0	**99**	div	

° calculation p. 20

RA**	50-150 %	RA**	50-150 %
Number in RA	**7**	Number in RA	**7**
Percent in RA	**70**	Percent in RA	**70**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:
For the spiking level sample 70% (7) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A also 70% (7) of the obtained recovery rates were within the recommended range.

4.2.2 ELISA Results: Milk (as Milk Protein)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
9	positiv	>10	negativ	0	1/1 (100%)	AQ	
17	positiv	18,0	positiv	2,00	1/1 (100%)	RS-F	
12	positiv	>8,3	negativ	<0,83	1/1 (100%)	VT	result converted °

° calculation p. 20

	Sample A		Sample B	
Number positive	3		1	
Number negative	0		2	
Percent positive	100		33	
Percent negative	0		67	
Consensus value	positiv		none	

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

Comments:
The consensus value for sample A was in qualitative agreement with the spiking of sample A. For sample B there was one positive result.

Quantitative valuation of results: Sample A

No statistical evaluation was performed, due to the low number of results.

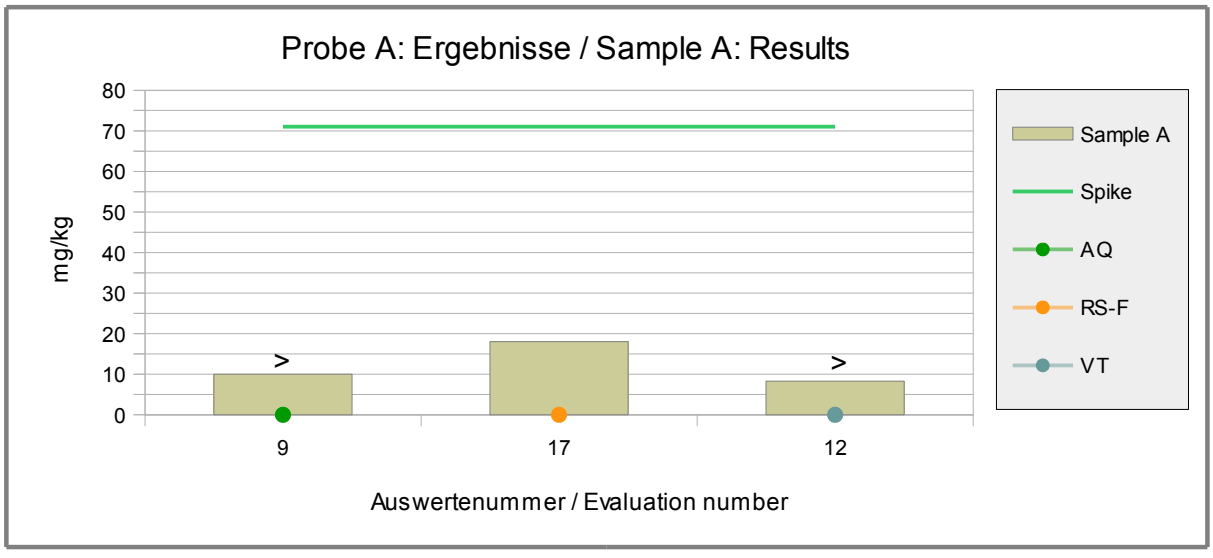


Abb./Fig. 13: ELISA Results Milk (as milk protein)
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Quantitative valuation of results: Spiking level sample

No statistical evaluation was performed, due to the low number of results.

Evaluation number	Milk protein	Milk protein	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
9	positive	>10		AQ	
17	positive	71		RS-F	
12	positive	>8,3		VT	result converted °

° calculation p. 20

Number positive	3
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

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

Comments:
There were exclusively positive results for the spiking level.

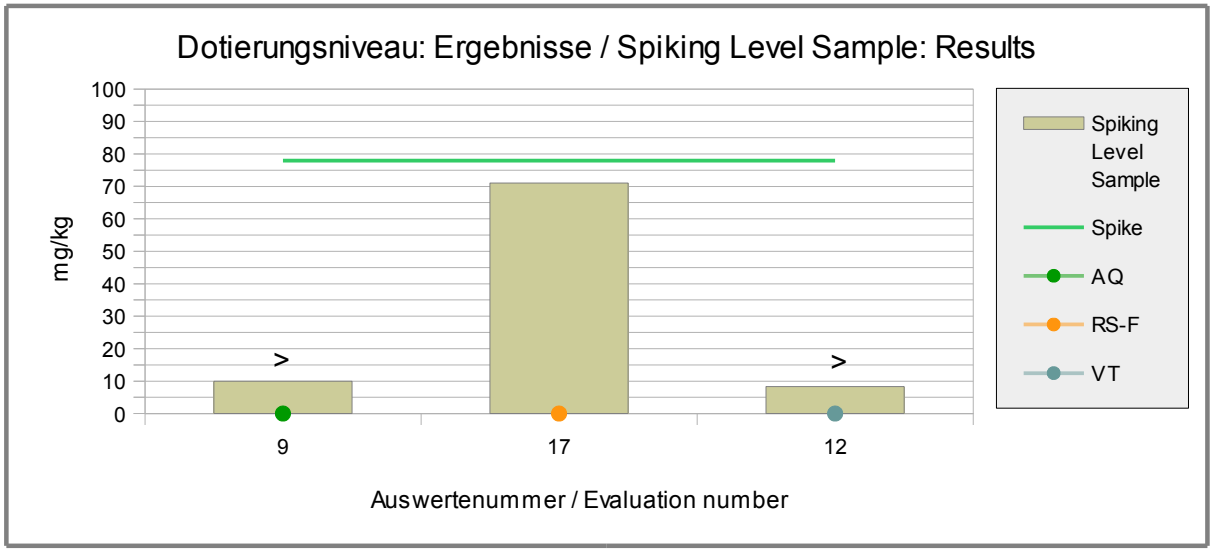


Abb./Fig. 14: ELISA Results Milk (as milk protein)
green line = Spiking level (Spike)
round symbols = Applied methods (see legend)

Recovery Rates for Milk (as Milk Protein): Spiking level sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
9	>10		>10		AQ	
17	71	**91**	18	25	RS-F	
12	>8,3		>8,3		VT	result converted °

° calculation p. 20

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

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

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
One participant submitted quantitative results by ELISA and obtained a recovery rate within the range of the AOAC-recommendation of 50-150% for the spiking level sample. For the spiked food matrix sample A the recovery rate was below the recommended range.

4.3 Proficiency test Wheat (Gluten/Wheat)

4.3.1 ELISA Results: Gluten

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
15a	positive	60,0	negative	<3,12	2/2 (100%)	EF-Q	
15b	positive	60,0	positive	3,70	1/2 (50%)	EF-R5	
18	positive	114	negative	< 0.6	2/2 (100%)	IL	
1	positive	42,7	negative	N/A	2/2 (100%)	RS	
2	positive	73,7	positive	7,00	1/2 (50%)	RS	
3	positive	62,6	negative		2/2 (100%)	RS	
5	positive	43,1	negative	<5	2/2 (100%)	RS	
6	positive	79,8	negative		2/2 (100%)	RS	
7	positive	57,5	negative	<5	2/2 (100%)	RS	
8	positive		negative		2/2 (100%)	RS	
10	positive	51,6	negative		2/2 (100%)	RS	
11	positive	50,7	negative	< 5,0	2/2 (100%)	RS	
12	positive	67,0	negative	<5	2/2 (100%)	RS	
14	positive	43,3	negative	<5	2/2 (100%)	RS	
16	positive	62,6	positive	5,85	1/2 (50%)	RS	
17	positive	19,0	negative	0	2/2 (100%)	RS	
15c	positive	51,0	positive	<5	1/2 (50%)	RS	
9	positive	27,2	negative	0	2/2 (100%)	VT-R5	
13	positive	64,0	negative	< 5	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	19		4	
Number negative	0		15	
Percent positive	100		21	
Percent negative	0		79	
Consensus value	positive		negative	

Methods:

EF-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT-R5 = Veratox, Neogen
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. Four positive results each < 10 mg/kg or below the limit of quantification were obtained for sample B.

Quantitative valuation of results: Sample A

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
15a	60,0	0,26		EF-Q	
15b	60,0	0,26		EF-R5	
18	114	4,1		IL	
1	42,7	-1,0	-0,90	RS	
2	73,7	1,2	1,4	RS	
3	62,6	0,45	0,55	RS	
5	43,1	-0,94	-0,87	RS	
6	79,8	1,7	1,8	RS	
7	57,5	0,08	0,18	RS	
8				RS	
10	51,6	-0,34	-0,25	RS	
11	50,7	-0,40	-0,32	RS	
12	67,0	0,76	0,87	RS	
14	43,3	-0,93	-0,86	RS	
16	62,6	0,44	0,55	RS	
17	19,0	-2,7	-2,6	RS	
15c	51,0	-0,38	-0,29	RS	
9	27,2	-2,1		VT-R5	
13	64,0	0,55		div	

Methods:

EF-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT-R5 = Veratox, Neogen
div = not indicated / other method

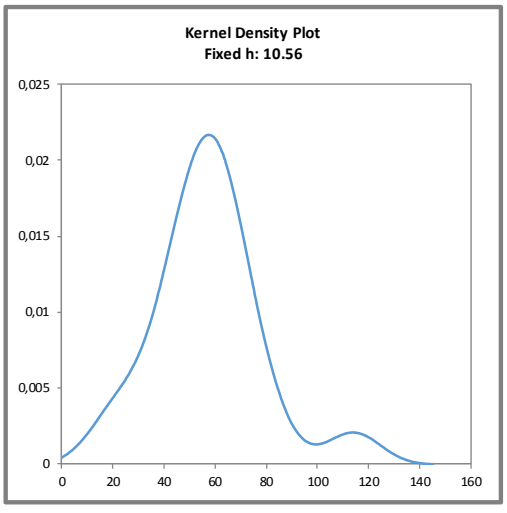


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 side peak at 114 mg/kg, due to a single result (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	18	13
Number of outliers	-	0
Mean	57,2	54,2
Median	58,8	51,6
Robust Mean (X_{pt})	**56,3**	**55,1**
Robust standard deviation (S*)	**16,4**	**15,0**
Target range:		
Target standard deviation σ_{pt}	**14,1**	**13,8**
lower limit of target range	**28,2**	**27,5**
upper limit of target range	**84,5**	**82,6**
Quotient S/σ_{pt}*	*1,2*	*1,1*
Standard uncertainty $U_{(Xpt)}$	*4,84*	*5,21*
Results in the target range	*15*	*12*
Percent in the target range	*83*	*92*

Methods:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results without clear method-dependent differences.

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

The assigned values X_{pt} (robust means) of the evaluations were 149% and 146% of the spiking level of gluten to sample A and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for gluten", p.50).

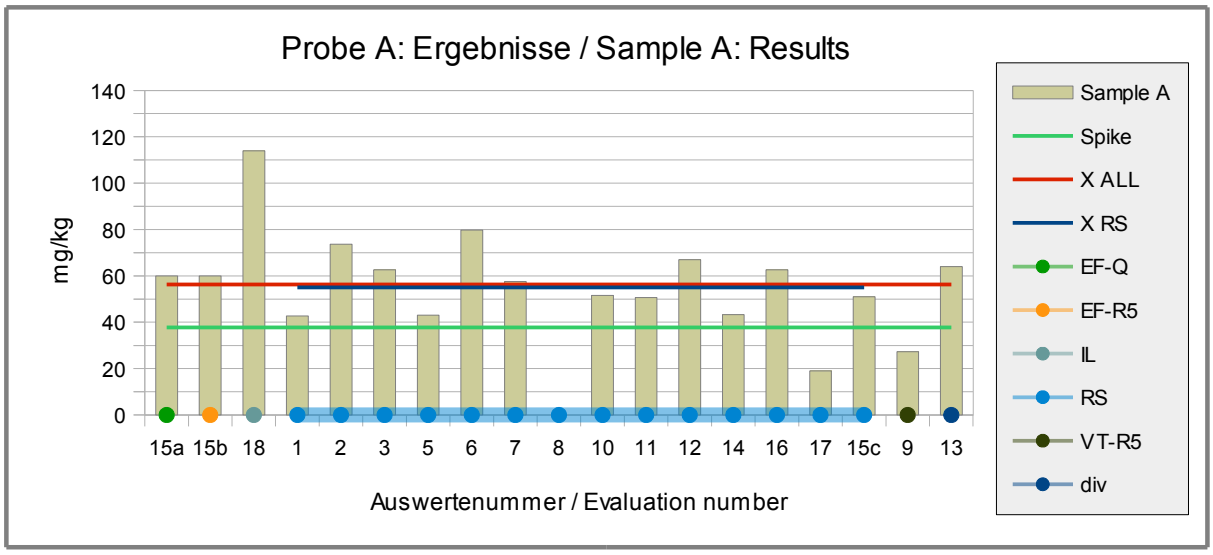


Abb./Fig. 16: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

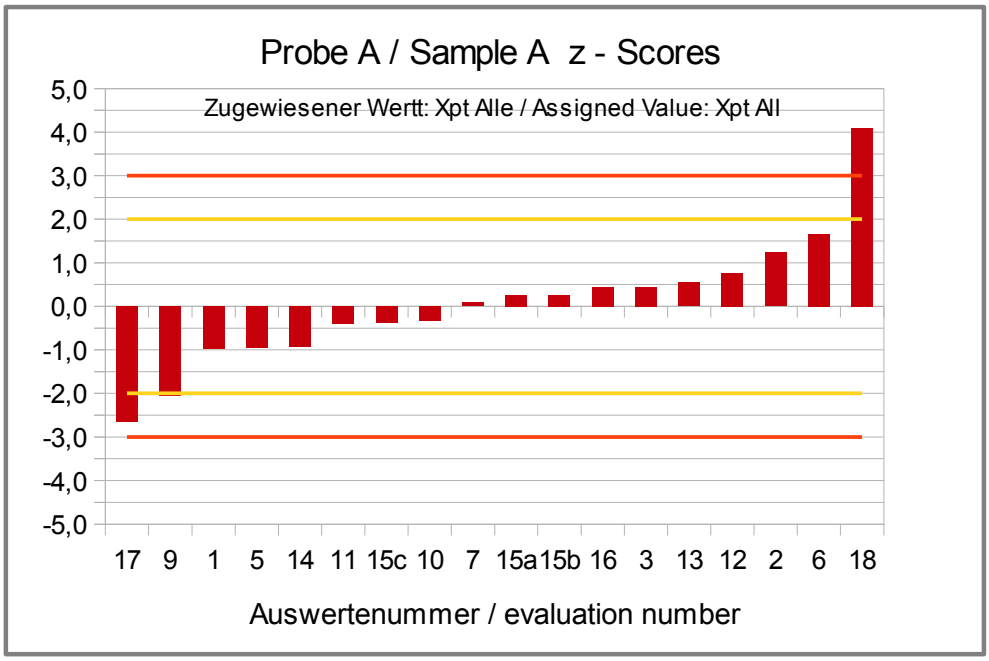


Abb./Fig. 17:
z´-Scores (ELISA Results Gluten) Assigned value robust mean of all results

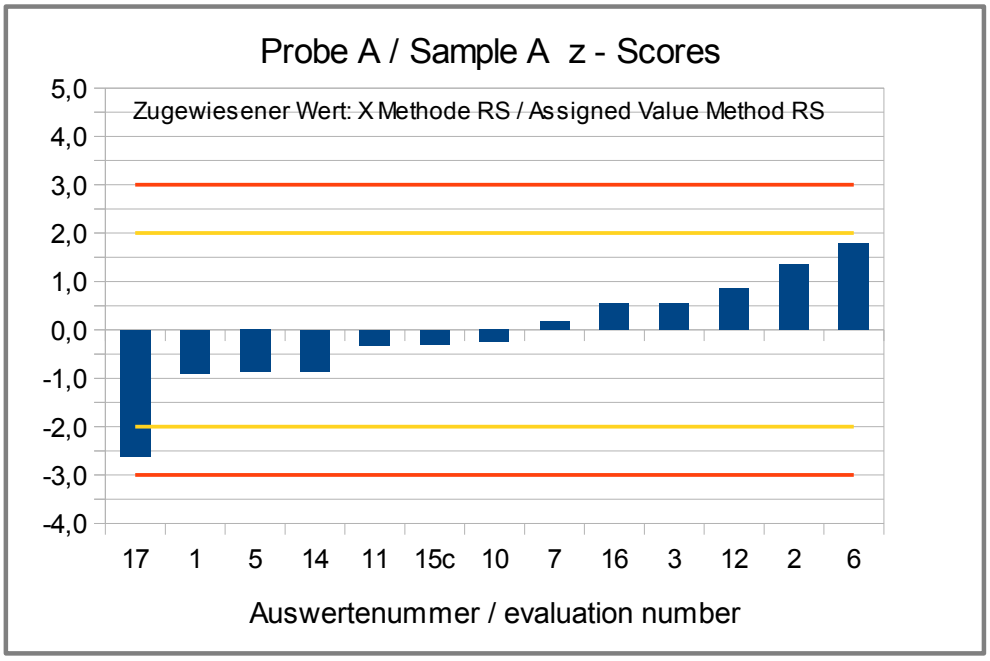


Abb./Fig. 18:
z-Scores (ELISA Results Gluten) Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Quantitative valuation of ELISA results: Spiking level sample

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
15a	63,0	1,2		EF-Q	
15b	48,0	-0,02		EF-R5	
18	158	9,1		IL	
1	49,0	0,06	0,31	RS	
2	55,7	0,62	0,91	RS	
3	49,8	0,13	0,38	RS	
5	31,3	-1,4	-1,2	RS	
6	67,5	1,6	1,9	RS	
7	46,6	-0,14	0,10	RS	
8		-4,0		RS	
10	45,3	-0,24	-0,01	RS	
11	46,8	-0,12	0,12	RS	
12	43,0	-0,44	-0,21	RS	
14		-4,0	-4,0	RS	
16	39,8	-0,70	-0,50	RS	
17	14,0	-2,8	-2,8	RS	
15c	47,0	-0,10	0,14	RS	
9	28,0	-1,7		VT-R5	
13	63,0	1,2		div	

Methods:

EF-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins

EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins

IL = Immunolab

RS = Ridascreen®, R-Biopharm

VT-R5 = Veratox, Neogen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

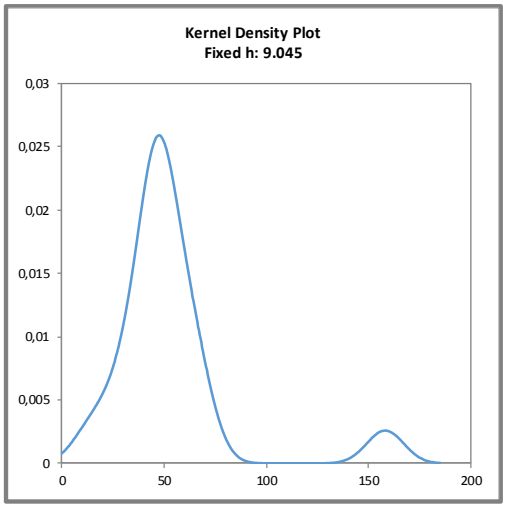


Abb. / Fig. 19:

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 side peak at 158 mg/kg, due to a single result (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	17	12
Number of outliers	-	-
Mean	52,7	44,7
Median	47,0	46,7
Robust Mean (X_{pt})	**48,3**	**45,4**
Robust standard deviation (S*)	**14,5**	**9,71**
Target range:		
Target standard deviation σ_{pt}	**12,1**	**11,4**
lower limit of target range	**24,1**	**22,7**
upper limit of target range	**72,4**	**68,1**
Quotient S/σ_{pt}*	*1,2*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*4,41*	*3,50*
Results in the target range	*15*	*11*
Percent in the target range	*88*	*92*

Methods:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results without clear method-dependent differences.

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

The assigned values X_{pt} (robust means) of the evaluations were 134% and 126% of the spiking level of gluten to the spiking level sample and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for gluten", p.50).

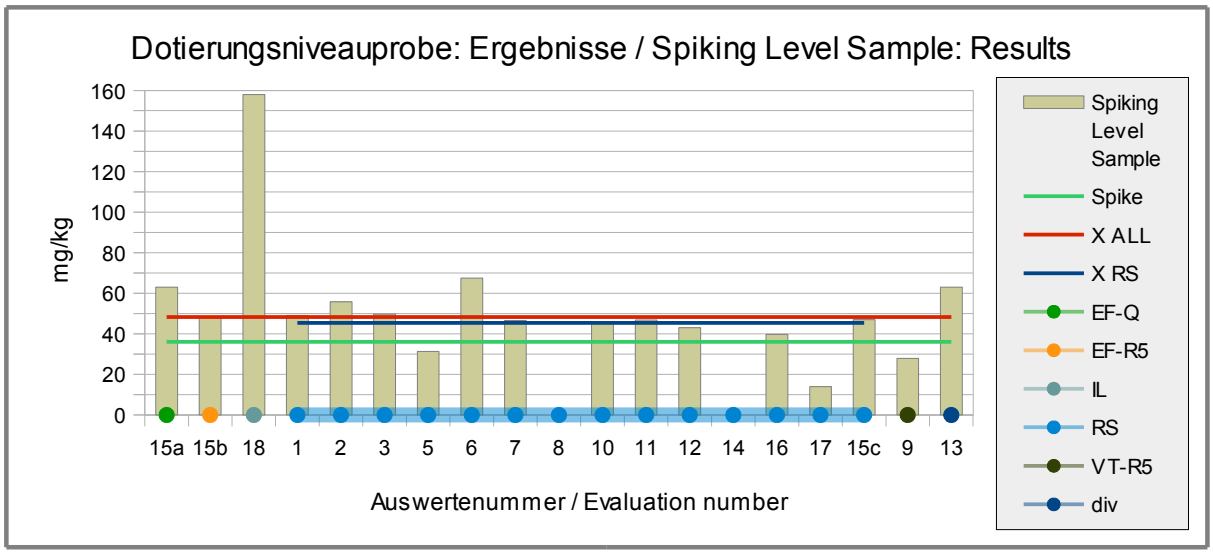


Abb./Fig. 20: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

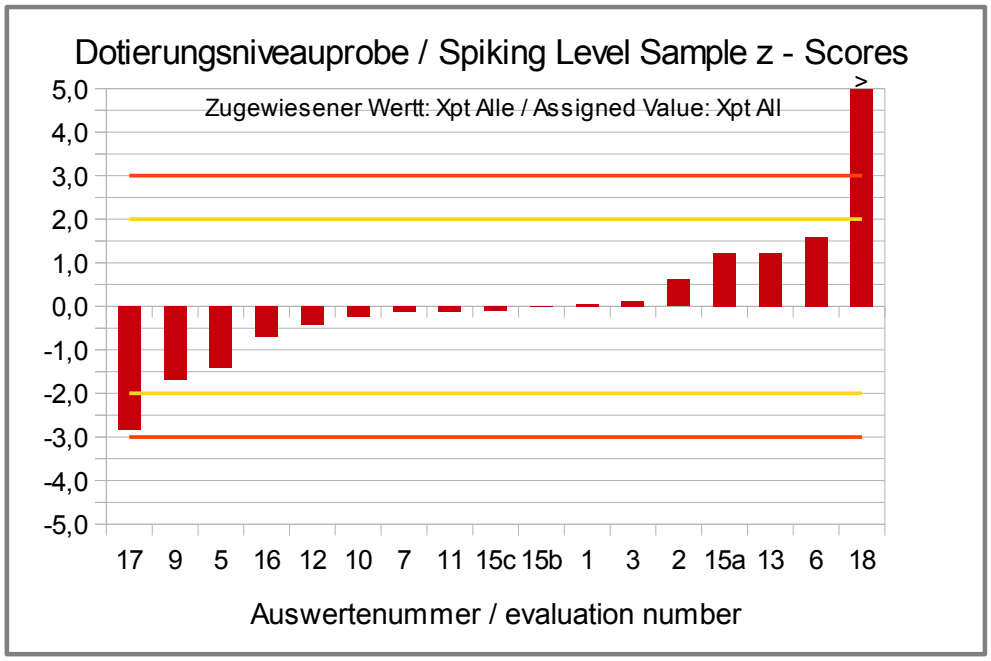


Abb./Fig. 21:
z-Scores (ELISA Results Gluten) Assigned value robust mean of all results

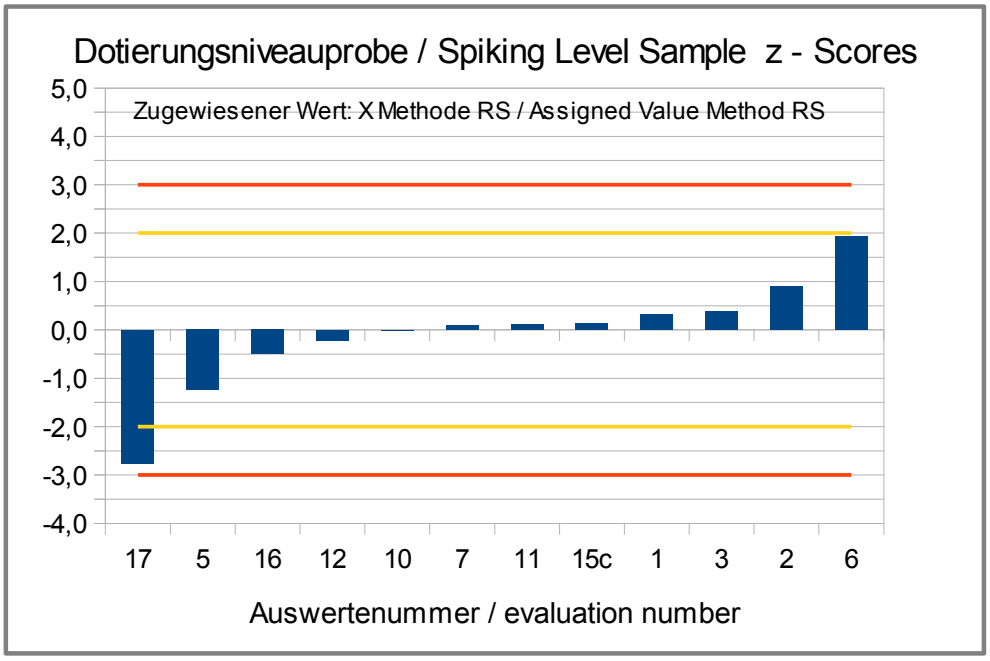


Abb./Fig. 22:
z-Scores (ELISA Results Gluten) Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Recovery Rates for Gluten:
Spiking level sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
15a	63,0	175	60,0	159	EF-Q	
15b	48,0	**133**	60,0	159	EF-R5	
18	158	438	114	302	IL	
1	49,0	**136**	42,7	**113**	RS	
2	55,7	154	73,7	195	RS	
3	49,8	**138**	62,6	166	RS	
5	31,3	**87**	43,1	**114**	RS	
6	67,5	187	79,8	212	RS	
7	46,6	**129**	57,5	153	RS	
8					RS	
10	45,3	**125**	51,6	**137**	RS	
11	46,8	**130**	50,7	**134**	RS	
12	43,0	**119**	67,0	178	RS	
14			43,3	**115**	RS	
16	39,8	**110**	62,6	166	RS	
17	14,0	39	19,0	**50**	RS	
15c	47,0	**130**	51,0	**135**	RS	
9	28,0	**77**	27,2	**72**	VT-R5	
13	63,0	175	64,0	170	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**11**	Number in RA	**8**
Percent in RA	**65**	Percent in RA	**44**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
EF-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins
EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT-R5 = Veratox, Neogen
div = not indicated / other method

Comments:
For the spiking level sample 65% (11) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 44% (8) of the obtained recovery rates were within the recommended range.

4.3.2 PCR Results: Gluten-containing Cereals (Wheat)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
9	positive		positive		-	4L	
15	positive		positive		-	div	

	Sample A		Sample B	
Number positive	2		2	
Number negative	0		0	
Percent positive	100		100	
Percent negative	0		0	
Consensus value	positive		positive	

Methods:
4L = 4LAB Diagnostics
div = not indicated / other method

Comments:
The consensus value of sample A was in qualitative agreement with the spiking of sample A. For the unspiked sample B, positive results were also obtained. By ELISA there were also some positive results for sample B with gluten contents of < 10 mg/kg.

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: β-Lactoglobulin

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
AQ	9	06.04.18	positive	>0,4	positive	0,0087	positive	>0,4	0,0015	0,01	0,15	beta-Lactoglobulin	AgraQuant ELISA β-Lactoglobulin COLAL1048, RomerLabs
BK	6	29.03.18	positive	14,8	negative		positive	18	2,5	2,5	20	beta-Lactoglobulin	BioKits BLG Assay Kit, Neogen
ES	1a	09.04.17	positive	8	negative	N/A	positive	9,3		0,1	31,2	beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
ES	7	21.03.	positive	19,6	negative	<0,1	positive	21,5	0,05	0,1	16,8	beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
ES	12		positive	>1	negative	<0,1	positive	>1		0,1		beta-Lactoglobulin	ELISA Systems Beta-Lactoglobulin ESMRDBLG-48
MI	1b	20.04.17	positive	36,6MP	negative	N/A	positive	48,3MP		0,312	50,6	Milk proteins	Morinaga BLG ELISA Kit
Mi	15	20.3.	positive	4,2	negative	<0,031	positive	4,7	0,031	0,031		beta-Lactoglobulin	Morinaga ßLac ELISA Kit II M2112
NL	3	27.04.18	-	1,8	negative		-	1,9	0,0015	0,01		beta-Lactoglobulin	nutriLinia® Allergen-ELISA BLG-E NC6035
RS	4	08.03.18	positive	3,28	negative	<2,63	positive	3,7	0,79	2,63	31	beta-Lactoglobulin	Ridascreen® β-Lactoglobulin R4901, R-Biopharm
RS	16	23.03.18	-	<5	-	<5	-	<5					r-biopharm RIDASCREEN ß-Lacotglobulin
RS-F	5	12.04.18	positive	7,1	negative	<0.167	positive	5,68	0,167	0,167	33,7	beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	10	21.03.-25.04.	positive	1,66	negative		positive	2,95	0,07	0,2		Protein	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
RS-F	13	20.03.18	detectable	5	negative	< 0,5	detectable	5,1				Protein	r-biopharm, beta-LG-Fast
RS-F	14		positive	2,24	negative	<0,5	-		0,19	0,5		beta-Lactoglobulin	Ridascreen® FAST β-Lactoglobulin R4902, R-Biopharm
ZL	2	11.04.18	positive	>0,5	positive	0,08	positive	>0,5	0,05	0,5	20	beta-Lactoglobulin	other: ZEULAB - PROTEON MILK

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

Continuation ELISA β-Lactoglobulin:

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	9	anti-beta-lactoglobulin		yes	Measurement range: 10 -400 ppb Cross reactivity to: sheep's milk 0,2%, goat's milk 0,002%, casein 0,02%.
BK	6	beta-Lactoglobulin		no	
ES	1a	β-Lactoglobulin	Manufacturer's extraction solution / 15 min / 60C	yes	
ES	7		As per kit instructions	yes	Sw elling sample became temporarily solid during extraction
ES	12			NO	
MI	1b	β-Lactoglobulin	Extraction 100 °C/10 mins	yes	
Mi	15	ß Lactoglobulin	As per kit instructions	yes	ß Lactoglobulin
NL	3			yes	
RS	4	Anti-BLG	w ashing buffer, 10 minutes, 50°C	No	
RS	16				R4901
RS-F	5	As per kit instructions	As per kit instructions	Yes	Samples solidified on addition of reagents
RS-F	10			yes	NEW: Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm
RS-F	13			no	
RS-F	14			yes	
ZL	2				

5.1.2 ELISA: Casein

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
AQ	6	21.03.18	positive	61	negative		positive	63,3	1	1	40	Casein	AgraQuant Casein CO-KAL 1200, RomerLabs
ES	12		positive	>10	negative	<1	positive	>10		1		Skimmed milk powder	ELISA Systems Casein ES-CASPRD-48
ES	1a	09.04.17	positive	130	negative	N/A	positive	75,6		1	30,7	Skimmed milk powder	ELISA Systems Casein ES-CASPRD-48
IL	9	09.04.18	positive	>6,0	negative	0	positive	>6,0	0,04	0,2	0,15	Casein	Immunolab Casein ELI-SA
IL	18	19.03.18	positive	87	negative	< 0.01	positive	75	0.04	0.2		Casein	Immunolab Casein ELISA
MI	1b	21.04.17	positive	56,5	negative	N/A	positive	106,7		0,312	45,1	Milk proteins	Morinaga Casein ELISA Kit
NL	3	27.04.18	-	59,5	negative		-	60,5	0,05	0,2		Casein	nutriLinia® Allergen-ELI-SA Casein-E NC-6031
RS-F	5	24.04.18	positive	38,36	negative	<2.5	positive	35,07	2,5	2,5	31,97	Casein	Ridascreen® FAST Ca-sein R4612, R-Biopharm
RS-F	10	21.03.-25.04.	positive	113,6	negative		positive	87,3	0,12	0,5		Protein	Ridascreen® FAST Ca-sein R4612, R-Biopharm
RS-F	13	20.03.18	detecta-ble	75	negative	< 0,5	detecta-ble	103				Protein	r-Biopharm, Casein-Fast
RS-F	14		positive	>13,5	negative	<0,5	-		0,12	1		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
VT	7	21.03.	positive	189,8	negative	<2,5	positive	206,8	1	2,5	22	Casein	Veratox Casein Allergen, Neogen
div	15	20.3.	positive	56	negative	<0,25	positive	67	0,25	0		Casein	Selection Casein-Kits:

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6	Casein		no	
ES	12			NO	
ES	1a	Casein	Manufacturer's extraction solution / 15 min / 60C	yes	
IL	9	anti-casein		yes	Measurement range: 0,2-6ppm Cross Reaktivität to: sheep's milk 1,2%, goat's milk 1,1%.
IL	18				
MI	1b	Casein	Extraction 100 °C/10 mins	yes	
NL	3			yes	
RS-F	5	As per kit instructions	As per kit instructions	Yes	Samples solidified on addition of re-agents
RS-F	10			yes	
RS-F	13			no	
RS-F	14			yes	
VT	7		As per kit instructions	yes	Sw elling sample became temporarily so-lid during extraction
div	15	Casein from cow 's milk	As per kit instructions	yes	Casein

5.1.3 ELISA: Milk Protein

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
AQ	9	24.04.18	positive	>10	negative	0	positive	>10	0,05	0,4	0,15	Milk proteins, total	AgraQuant ELISA Milk COKAL2448, RomerLabs
RS-F	17		positive	18	positive	2	positive	71	10	20	50	Milk proteins, total	Ridascreen® FAST Milk R4652, R-Biopharm
VT	12		positive	>25	negative	<2,5	positive	>25		3		Skimmed milk powder	Veratox Total Milk Allergen, Neogen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	9	anti-bovine milk		yes	Measurement range: 0,4 - 10 ppm Cross reactivity to: sheep's milk 0,94%, goat's milk 0,01%.
RS-F	17			no	
VT	12			YES	

5.1.4 ELISA: Gluten

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
EF-Q	15a	27.3.	-	60	negative	<3,12	positive	63	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 Quick 30.GL2.K2
EF-R5	15b	27.3.	-	60	positive	3,7	positive	48	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2
IL	18	19.03.18	positive	114	negative	< 0.6	positive	158	0.06	4		Gluten	Immunolab Gliadin/Gluten ELISA
RS	1	09.04.18	positive	42,7	negative	N/A	positive	49		5	41,2	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	2	19.04.18	positive	73,7	positive	7	positive	55,74	5	80		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	3	27.04.18	-	62,6	negative		-	49,8	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	5	07.04.18	positive	43,06	negative	<5	positive	31,28	5	5	24,57	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	6	24.04.18	positive	79,8	negative		positive	67,5	5	5	25	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	7	21.03.	positive	57,5	negative	<5	positive	46,6	1	5	23	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8	26.03.18	positive		negative		positive						Ridascreen® Gliadin R7001, R-Biopharm
RS	10	21.03.-25.04.	positive	51,6	negative		positive	45,3	1	5		Protein	Ridascreen® Gliadin R7001, R-Biopharm
RS	11	09.04.18	positive	50,68	negative	< 5,0	positive	46,82				Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	12		positive	67	negative	<5	positive	43		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	14		positive	43,29	negative	<5	-		3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	16	23.03.18	-	62,58	-	5,85	-	39,75					r-biopharm RIDASCREEN Gliadin
RS	17		positive	19	negative	0	positive	14	10	20	50	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	15c	16.3.	-	51	positive	<5	positive	47	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
VT-R5	9	18.04.18	positive	27,23	negative	0	positive	27,95	3	5	0,15	Gluten	Veratox Gliadin R5, Neogen
div	13	20.03.18	detectable	64	negative	< 5	detectable	63				Protein	R-Biopharm, Sandwich

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

Continuation ELISA: Gluten

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
EF-Q	15a	R5, detects prolamins from w heat, rye and barley	As per kit instructions	yes	
EF-R5	15b	R5, detects prolamins from w heat, rye and barley	As per kit instructions	yes	
IL	18				
RS	1	Gluten (R5)	Cocktail solution / 40 min / 50C	yes	
RS	2	Monoclonal antivody R5	Cocktail (patented) / 40 min / 50°C. Ethanol solution (80%) / 1h /20-25°C		
RS	3			yes	
RS	5	As per kit instructions	As per kit instructions	Yes	Samples solidified on addition of reagents
RS	6	R5, detects prolamins from w heat, rye and barley		no	
RS	7		As per kit instructions	yes	Sw elling sample became temporarily solid during extraction
RS	8	R5		ja	
RS	10			ja	
RS	11	Monoclonal R5	80% ethanol / 1h / room temperature/	yes	LAB_AR results
RS	12	R5		YES	
RS	14			yes	
RS	16		Extraction w ith cocktail solution		R7001
RS	17			no	
RS	15c	R5, detects prolamins from w heat, rye and barley	As per kit instructions	yes	non-quantifiable traces betw een LOD and LOQ
VT-R5	9	anti-R5		yes	Measurement range: 5-80 ppm, Specificity: The fraction of gluten from w heat, rye, barley. No cross reactivity to: oats, corn, rice, soy, millet, teff, buckw heat, quinoa and amaranth.
div	13	R5		no	

5.1.5 PCR: Gluten-containing Cereals

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit + Manufacturer
4L	9	23.04.18	positive		positive		positive					Gluten	4LAB Diagnostics
div	15	16.3.	positive		positive		positive		4			gluten-containing cereals DNA	Eur F Res Tech 212 (2001) 228ff, mod.

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

Meth. Abk.	Auswerte-nummer	Spezifität	Hinweise zur Methode (Extraktion und Bestimmung)	Methode akkreditiert ISO/IEC 17025	Sonstige Hinweise
		Target-Sequenz / -DNA	z.B. Extraktion / Enzyme / Clean-Up / Real Time PCR / Gelelektrophorese / Cyclen	ja/nein	
4L	9			yes	test system for the qualitative detection of gluten-containing cereal DNA in food products by PCR Real Time
div	15		CTAB / Proteinase K / Promega Wizard DNA CleanUp / PCR / Gel electrophoresis / 45 cycles	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 03-2018 Sample A

Weight whole sample	2,78	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	60	23,8
2	4,99	59	23,6
3	5,06	67	26,5
4	5,01	62	24,8
5	5,06	69	27,3
6	4,97	71	28,6
7	5,08	65	25,6
8	5,08	72	28,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,6	Particles
Standard deviation	4,87	Particles
χ^2 (CHI-Quadrat)	2,53	
Probability	**92**	%
Recovery rate	125	%

Normal distribution		
Number of samples	8	
Mean	26,1	mg/kg
Standard deviation	1,94	mg/kg
rel. Standard deviaton	7,43	%
Horwitz standard deviation	9,80	%
HorRat-value	**0,76**	
Recovery rate	125	%

Microtracer Homogenitätstest
DLA 03-2018 Spiking Level Sample

Weight whole sample	1,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	27,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,10	90	35,3
2	5,10	97	38,0
3	5,03	83	33,0
4	4,98	87	34,9
5	5,03	88	35,0
6	5,00	81	32,4
7	4,97	81	32,6
8	5,07	102	40,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	88,6	Particles
Standard deviation	6,93	Particles
χ^2 (CHI-Quadrat)	3,79	
Probability	**80**	%
Recovery rate	129	%

Normal distribution		
Number of samples	8	
Mean	35,2	mg/kg
Standard deviation	2,75	mg/kg
rel. Standard deviaton	7,82	%
Horwitz standard deviation	9,36	%
HorRat-value	**0,84**	
Recovery rate	129	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter (1st letter):

PT number	***DLA 03-2018***
PT name	***Allergens III: β-Lactoglobulin, Casein and Gluten in Infant Food with "Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *Infant food (pap powder)/ ingredients: sorghum, rice, maize and buckwheat flour, thiamine and, potato powder, other food additives and allergenic foods skimmed milk powder and wheat flour (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods (skimmed milk powder and wheat flour)*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* ***β-Lactoglobulin, Casein*** *and/or* ***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 the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest April 27th 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. 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
		ITALY
		CANADA
		ITALY
		SPAIN
		Germany
		AUSTRIA
		Germany
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		POLAND
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		SWITZERLAND
		Germany
		Germany
		GREAT BRITAIN
		Germany
		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.]

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