Evaluation Report
proficiency test

DLA 02/2019

Allergens II:

Lupin and Wheat (Gluten)

in "gluten-free" Bread

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

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Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

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

The test material of the food matrix samples is a bread produced with a customary "gluten-free" bread bake mixture. The bread was bake at 190°C for 60 min and afterwards dried for approx. 10 h at 40-50°C. The basic composition of samples A and 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 B** was produced as follows:
An additional ingredient was a bread baked (190°C, 60 min) with the spiking material containing the allergenic ingredients lupin and wheat (mesh <500 µm). After drying (40°C, 10 h), crushing, sieving (mesh <1,5 mm) and homogenization, this ingredient was added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added and homogenized.

For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Bread, "gluten-free" (baked 190°C, 90 min) **Bread Bake Mixture, "gluten-free"** Ingredients: Maize starch, linseed flour, buckwheat flour, pea bran, rice bran, psyllium plant fiber, sugar, thickener: guar gum, salt other ingredients: vegetable oil, yeast, salt (water) and allergenic ingredients (see below)	100 g/100g	99,4 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,4 g/100 g
Lupin: - as sweet lupin flour* - thereof 36,6% total protein**	-	 97,9 mg/kg 35,8 mg/kg	 58,9 mg/kg 21,6 mg/kg
Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) - as wheat flour* - thereof 10,1% total protein** - thereof gluten***	-	 582 mg/kg 58,8 mg/kg 50,6 mg/kg	 349 mg/kg 35,2 mg/kg 30,4 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	<0,6 g/100 g	<0,6 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=6,25 for lupin protein and F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 8,7% gluten in wheat flour [34, 35, 36])

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples B and the spiking level sample showed a probability of 92% and 65%. 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 0,63 and 1,2 respectively. Aufgrund der ausreichenden Wahrscheinlichkeit wurde der HorRat-Wert für Probe B akzeptiert. The HorRat value of sample B was accepted because of the sufficient probability. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

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

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

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

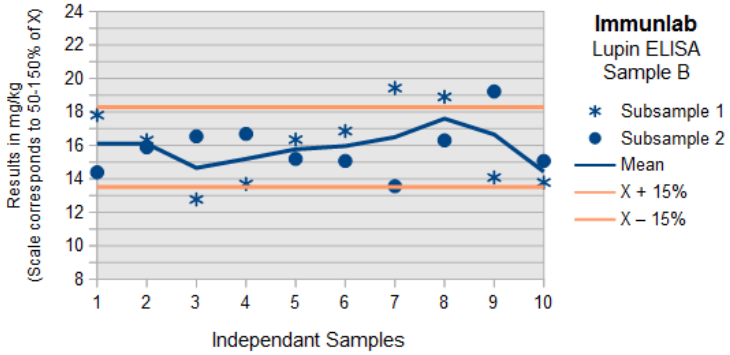
ELISA-Tests: Homogenität Lupine / Homogeneity Lupin

Immunolab Lupin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Lupin 15,9 ± 1,0 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	17,8	14,4	16,1
2	16,3	15,9	16,1
3	12,8	16,5	14,7
4	13,7	16,7	15,2
5	16,3	15,2	15,8
6	16,9	15,1	16,0
7	19,4	13,6	16,5
8	18,9	16,3	17,6
9	14,1	19,2	16,6
10	13,8	15,1	14,4

General average X	15,9	
SD of sample means Sx	0,95	6,0%
SD within-samples Sw	1,65	10,4%
SD between-samples Ss	< 0,95	<6,0%

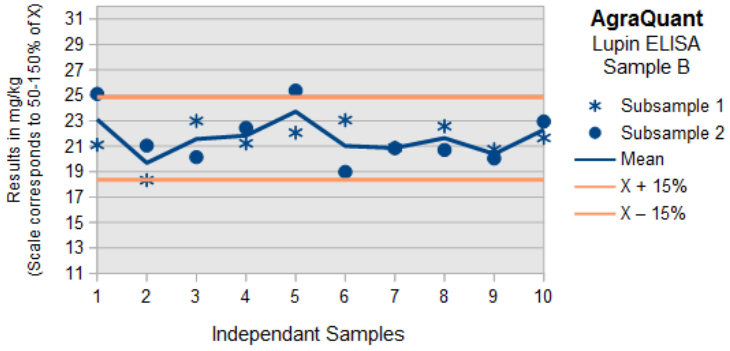


Romerlabs AgraQuant Lupin

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Lupin 21,6 ± 0,8 mg/kg

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

General average X	21,6	
SD of sample means Sx	1,22	5,6%
SD within-samples Sw	1,29	6,0%
SD between-samples Ss	0,80	3,7%



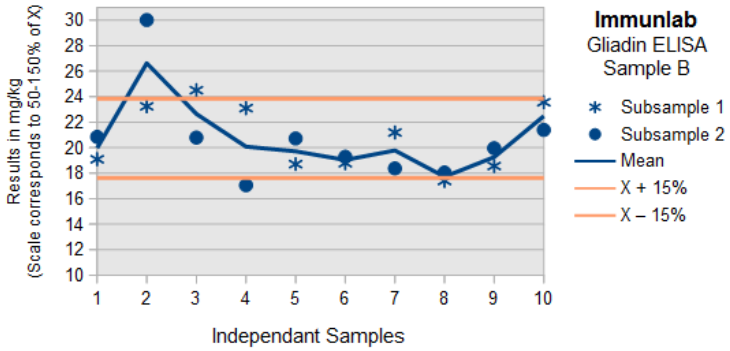
ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gliadin 20,7 ± 2,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	19,1	20,8	20,0
2	23,2	30,0	26,6
3	24,5	20,8	22,6
4	23,1	17,1	20,1
5	18,7	20,7	19,7
6	18,8	19,3	19,0
7	21,2	18,4	19,8
8	17,4	18,1	17,7
9	18,6	20,0	19,3
10	23,6	21,4	22,5

General average X	20,7	
SD of sample means Sx	2,55	12,3%
SD within-samples Sw	1,72	8,3%
SD between-samples Ss	2,24	10,8%

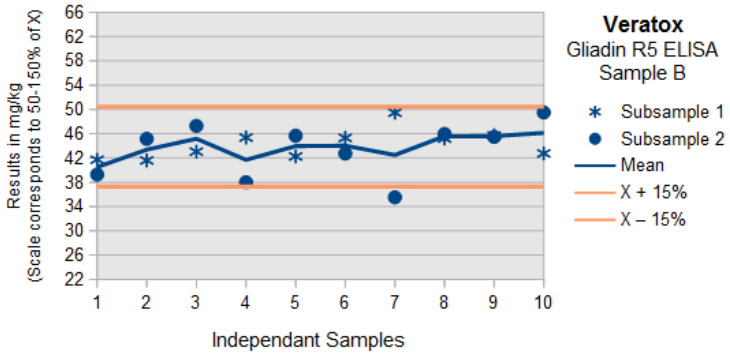


Neogen Veratox ELISA Gluten R5

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Gluten 43,8 ± 1,9 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	41,7	39,3	40,5
2	41,6	45,1	43,4
3	43,0	47,3	45,2
4	45,4	38,0	41,7
5	42,3	45,7	44,0
6	45,3	42,7	44,0
7	49,5	35,5	42,5
8	45,2	46,0	45,6
9	45,7	45,5	45,6
10	42,8	49,5	46,1

General average X	43,8	
SD of sample means Sx	1,86	4,2%
SD within-samples Sw	2,96	6,7%
SD between-samples Ss	<1,86	<4,2%

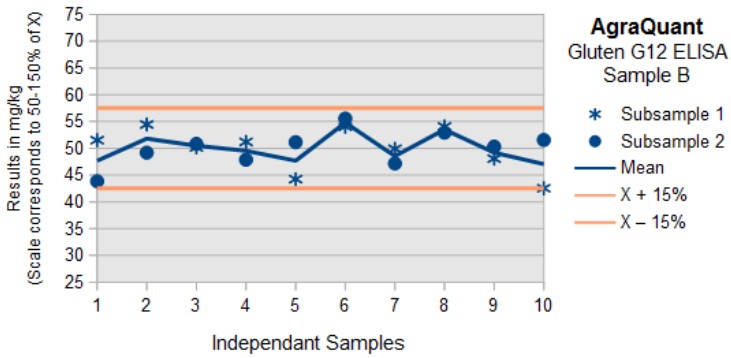


Romerlabs AgraQuant Gluten

Sample weights: 0,25 g (0,225 – 0,275 g)
Number of replicates: 2
Overall result: Gluten 50,0 ± 1,9 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	51,5	43,8	47,7
2	54,4	49,2	51,8
3	50,2	50,8	50,5
4	51,2	47,9	49,5
5	44,2	51,1	47,7
6	54,0	55,6	54,8
7	49,9	47,2	48,5
8	54,1	52,9	53,5
9	48,1	50,3	49,2
10	42,5	51,6	47,0

General average X	50,0	
SD of sample means Sx	2,61	5,2%
SD within-samples Sw	2,47	4,9%
SD between-samples Ss	1,94	3,9%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,20 (21,1°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 9th week of 2019. The testing method was optional. The tests should be finished at 12th April 2019 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters lupin and wheat in the range of mg/kg in the matrix of „gluten-free" bread. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The "spiking level sample" contains the allergens in a simple matrix in similar amounts without further processing.

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, 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.
13 out of 17 participants submitted their results in time. One participant submitted no results and three submitted late results in consultation with DLA.

3. Evaluation

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

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

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 21 – 45% 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} [32,33]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Lupin	Rice cookies	102 17,4 9,5	102 % 87 % 95 %	-	14,6% 26,5% 39,1%	23,0% 33,1% 42,6%	20,6% 27,3% 32,3%	rt-PCR multiplex ASU 18.00-22
Lupin	Wheat cookies Sauce powder	80,8 53,6	64,1 % 53,6 %	-	10,5% 23,9%	29,5% 48,0%	28,6% 44,8%	rt-PCR multiplex ASU 18.00-22
Wheat + Rye	Boiled saus-age (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σpt

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

3.8 Standard uncertainty and traceability

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

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

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

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

3.9 Figures

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

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

ELISA-results, which were given as **lupin flour** or **lupin**, were converted into total **lupin protein** using the analysed protein content of the raw material sweet lupin flour (see page 5). One PCR-result was submitted as lupin flour and evaluated as thus.

ELISA-results given as **gliadin** were converted into **gluten** multiplying the gliadin-content with the factor of 2.

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

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

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

4.1 Proficiency Test Lupin

4.1.1 ELISA Results: Lupin (as Lupin 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		
5	negative	0	positive	2,31	1/1 (100%)	BF	result converted °
12	negative	< 0,72	positive	4,38	1/1 (100%)	EF	result converted °
14	negative	< 0,72	positive	5,86	1/1 (100%)	EF	result converted °
1	negative		positive	5,40	1/1 (100%)	IL	result converted °
2	positive	< 0,72	positive	3,92	1/1 (100%)	IL	result converted °
9	negative	< 1	positive	8,00	1/1 (100%)	RS	
3	positive	2,40	positive	9,50	1/1 (100%)	RS-F	
6	negative	< 1	positive	6,75	1/1 (100%)	RS-F	
7	positive	1,01	positive	8,23	1/1 (100%)	RS-F	result converted °
8	positive	1,90	positive	11,9	1/1 (100%)	RS-F	
16	positive	1,30	positive	9,00	1/1 (100%)	RS-F	

° calculation p. 19

	Sample A		Sample B	
Number positive	5		11	
Number negative	6		0	
Percent positive	45		100	
Percent negative	55		0	
Consensus value	none		positive	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
The consensus value of sample B is in qualitative agreement with the spiking of sample B.
For sample A without spiked allergens no consensus value could be established, as there were less than 75% positive or negative results.
The positive results were just above the limits of quantification.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Lupin protein	z-Score Xpt_{ALL}	z-Score $Xpt_{PEAK\ 4}$	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]					
5	2,31	-2,6	-1,9		BF	result converted °
12	4,38	-1,4	0,01		EF	result converted °
14	5,86	-0,55	1,4		EF	result converted °
1	5,40	-0,82	0,94		IL	result converted °
2	3,92	-1,7	-0,42		IL	result converted °
9	8,00	0,71			RS	
3	9,50	1,6		0,19	RS-F	
6	6,75	-0,03		-1,0	RS-F	
7	8,23	0,84		-0,37	RS-F	result converted °
8	11,9	3,0		1,2	RS-F	
16	9,00	1,3		-0,04	RS-F	

° calculation p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

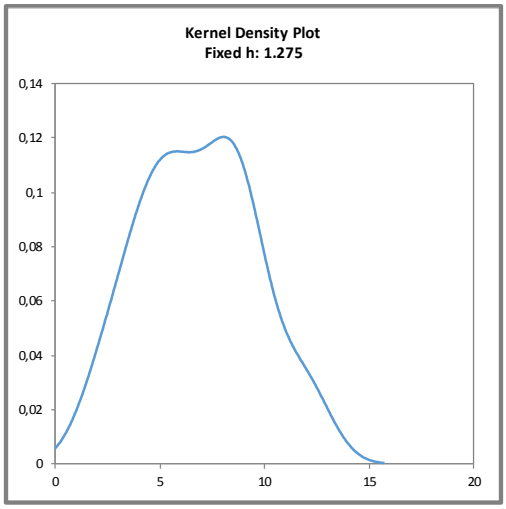


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a broad distribution of results with two indicated maxima at approximately 4-5 mg/kg and 8 mg/kg.

Characteristics: Quantitative evaluation ELISA Lupin Protein

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Meth. Peak 4** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{PEAK\ 4}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	11	5	5
Number of outliers	0	0	0
Mean	6,84	4,37	9,08
Median	6,75	4,38	9,00
Robust Mean (X_{pt})	**6,80**	**4,37**	**9,08**
Robust standard deviation (S*)	**3,09**	**1,58**	**2,14**
Target range:			
Target standard deviation σ_{pt}	**1,70**	**1,09**	**2,27**
lower limit of target range	**3,40**	**2,19**	**4,54**
upper limit of target range	**10,2**	**6,56**	**13,6**
Quotient S/σ_{pt}*	*1,8*	*1,4*	*0,94*
Standard uncertainty $U_{(Xpt)}$	*1,16*	*0,88*	*1,20*
Results in the target range	*9*	*5*	*5*
Percent in the target range	*82*	*100*	*100*

Methods:
Meth. Peak 4 = BF (BioFront), EF (Eurofins), IL (Immunolab)
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a distribution with two merging peaks. Since the second maximum can be assigned to the Ridascreen methods, a separate evaluation of the first maximum without Ridascreen methods ("Peak 4") was additionally performed.

The evaluation of all methods, of methods "peak 4" and of the method RS-F showed all a normal to low variability of results. The quotients S/σ_{pt} were below 2,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 19%, 12% and 25% of the spiking level of lupin to sample B and thus below the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of lupin protein" p.30).

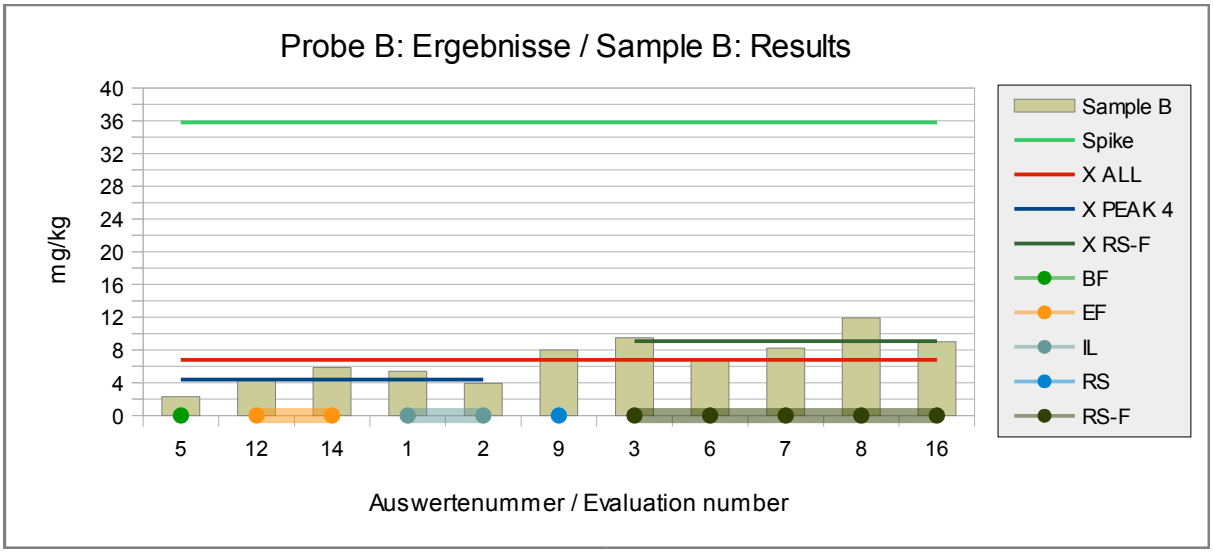


Abb./Fig. 2: ELISA Results Lupin Protein
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results methods peak 4
dark green line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

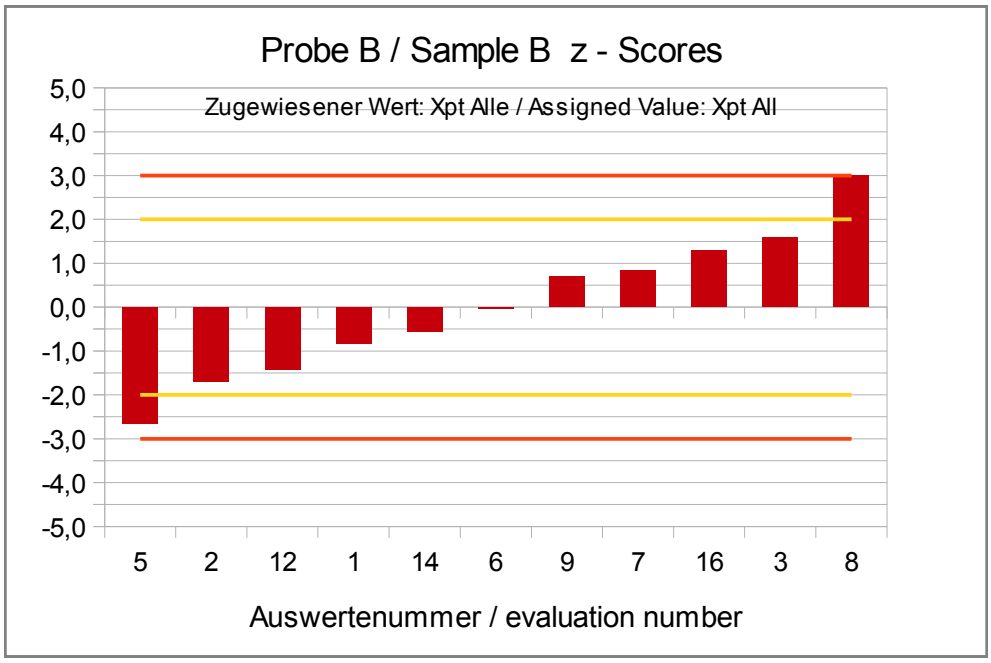


Abb./Fig. 3:
z-Scores (ELISA Results Lupin Protein)
Assigned value robust mean of all results

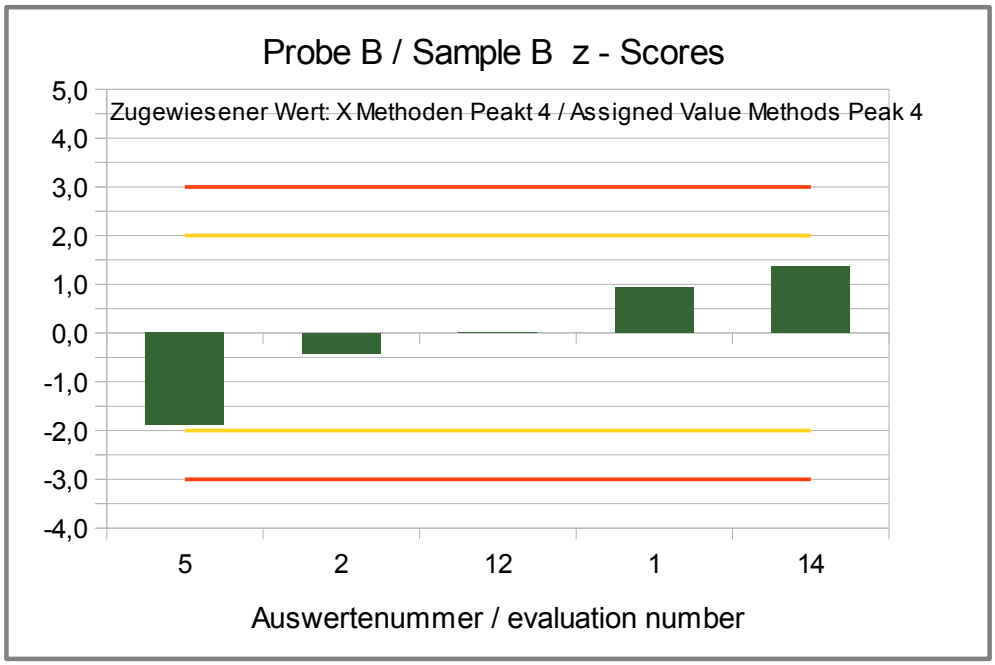


Abb./Fig. 4:
z-Scores (ELISA Results Lupin Protein)
Assigned value robust mean of methods peak 4 (BF, EF, IL)

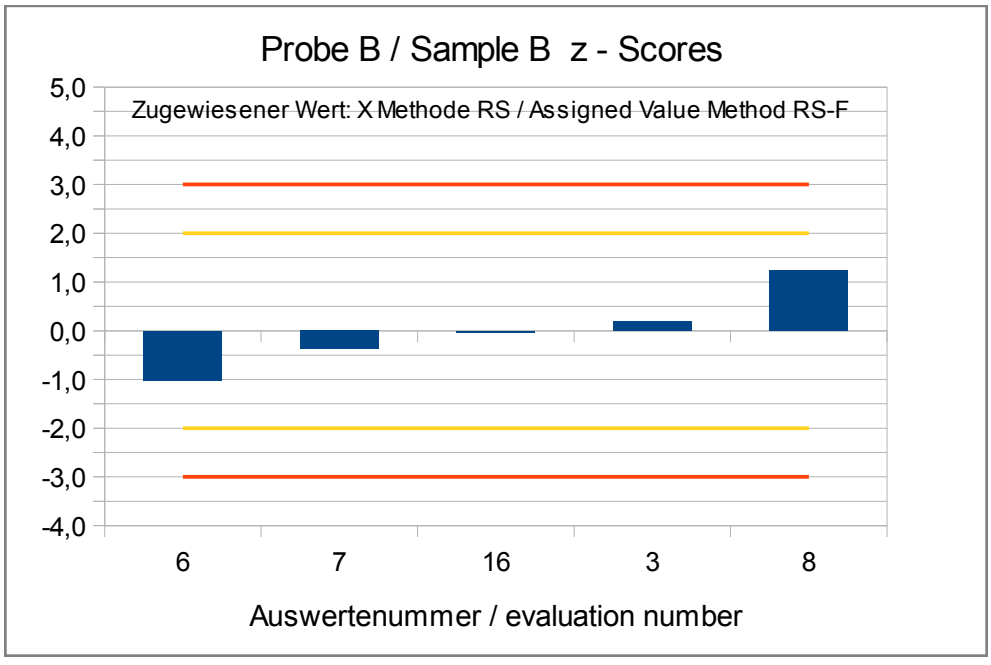


Abb./Fig. 5:
z-Scores (ELISA Results Lupin Protein)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Lupin Protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
5	15,3	-2,1		BF	result converted °
12	47,6	2,0		EF	result converted °
14	32,9	0,16		EF	result converted °
1	40,0	1,0		IL	result converted °
2	25,6	-0,78		IL	result converted °
9				RS	
3	18,1	-1,7	-1,7	RS-F	
6	34,9	0,40	0,43	RS-F	
7	31,3	-0,05	-0,03	RS-F	result converted °
8	38,0	0,79	0,83	RS-F	
16	33,0	0,16	0,19	RS-F	

° calculation p. 19

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

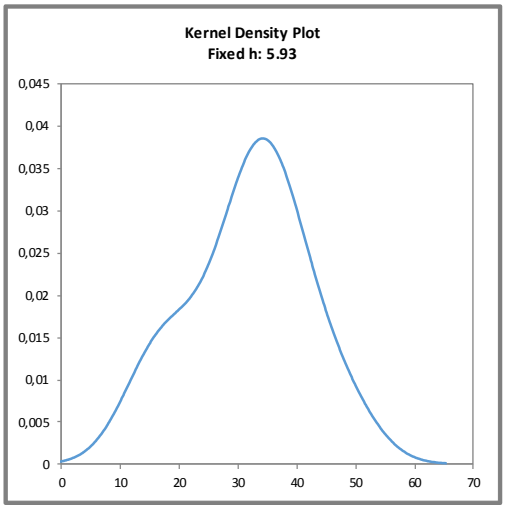


Abb. / Fig. 6:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a shoulder at approx. 15-20 mg/kg.

Characteristics: Quantitative evaluation ELISA Lupin Protein

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	10	5
Number of outliers	0	0
Mean	31,7	31,1
Median	33,0	33,0
Robust Mean (X_{pt})	**31,7**	**31,5**
Robust standard deviation (S*)	**11,1**	**7,5**
Target range:		
Target standard deviation σ_{pt}	**7,91**	**7,89**
lower limit of target range	**15,8**	**15,8**
upper limit of target range	**47,5**	**47,3**
Quotient S/σ_{pt}*	*1,4*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*4,39*	*4,20*
Results in the target range	*8*	*5*
Percent in the target range	*80*	*100*

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

Comments to the statistical characteristics and assigned values:

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

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

The robust means of the evaluations were 147% and 146% of the spiking level of lupin to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of lupin protein" p.30).

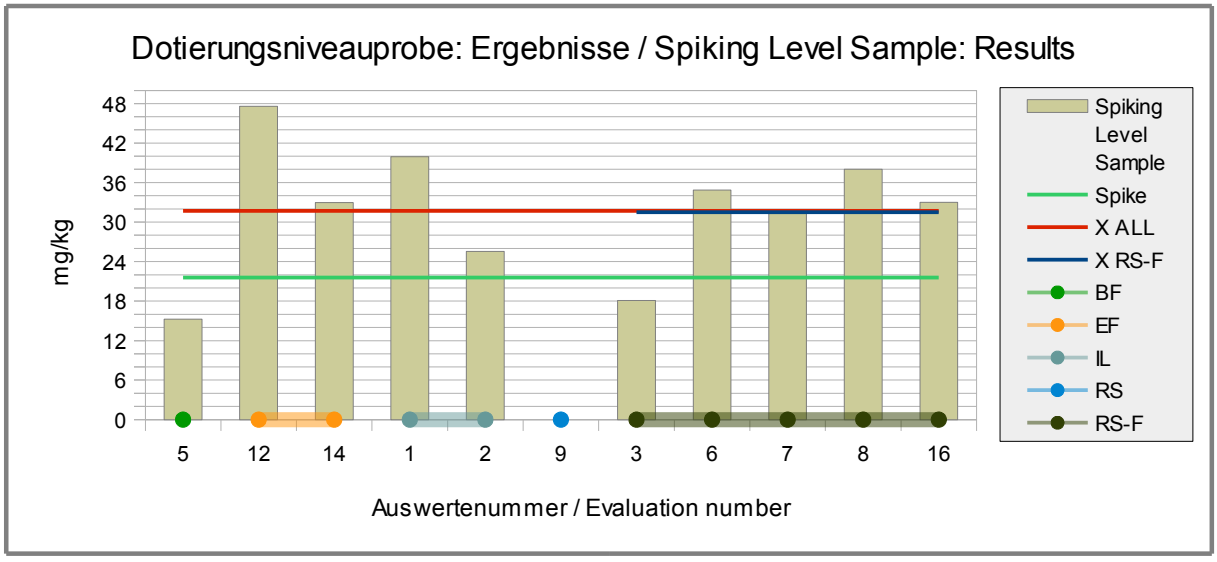


Abb./Fig. 7: ELISA Results Lupin Protein
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

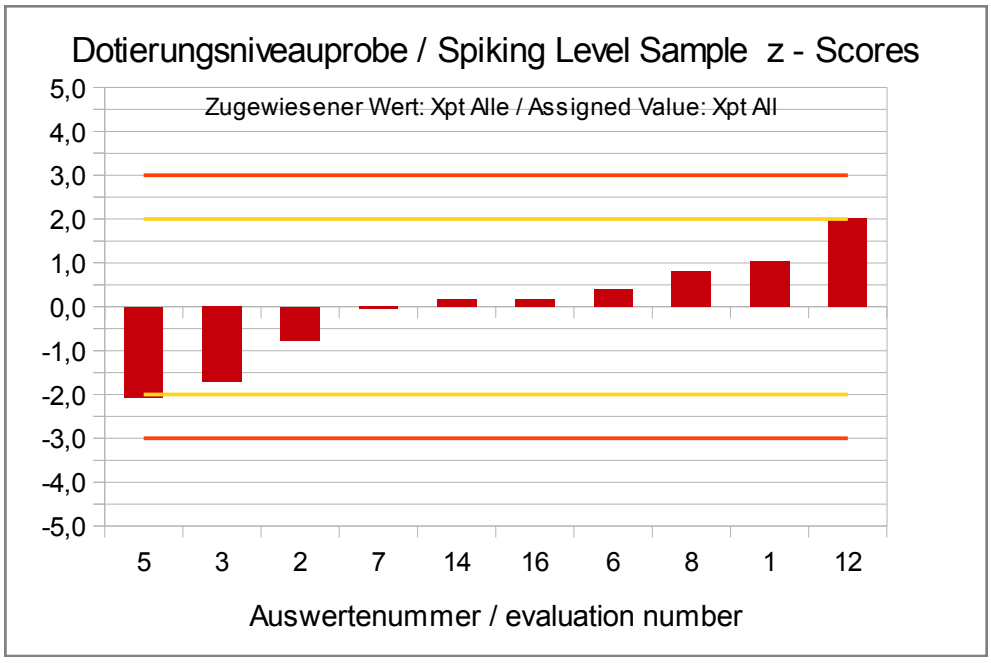


Abb./Fig. 8:
z'-Scores (ELISA Results Lupin Protein)
Assigned value robust mean of all results

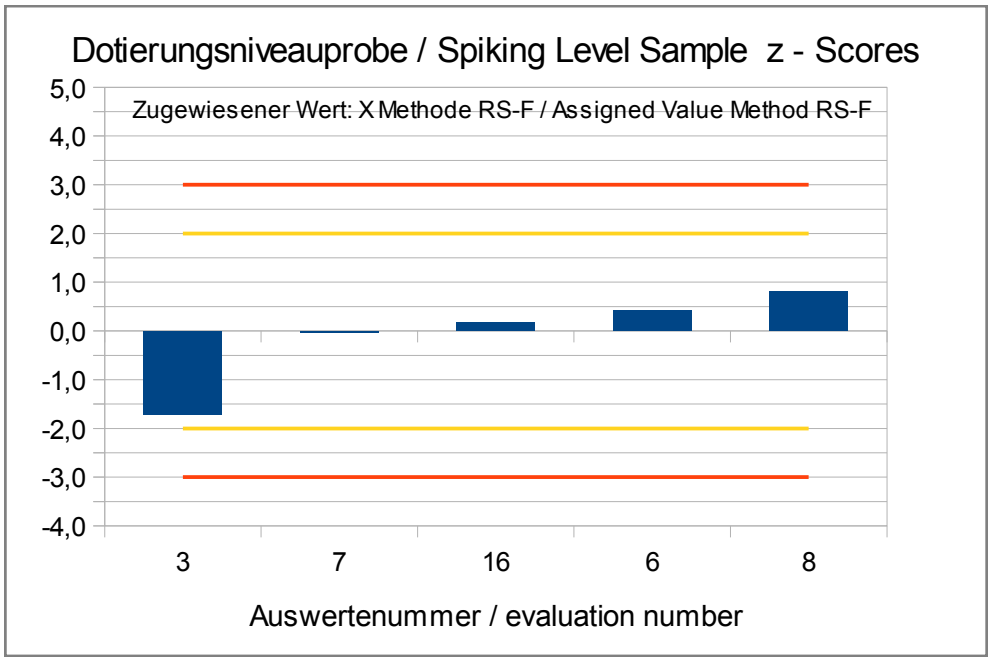


Abb./Fig. 9:
z-Scores (ELISA Results Lupin Protein)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates ELISA for Lupin Protein:
Spiking level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
5	15,3	**71**	2,31	6,5	BF	result converted °
12	47,6	220	4,38	12	EF	result converted °
14	32,9	153	5,86	16	EF	result converted °
1	40,0	185	5,40	15	IL	result converted °
2	25,6	**118**	3,92	11	IL	result converted °
9			8,00	22	RS	
3	18,1	**84**	9,50	27	RS-F	
6	34,9	161	6,75	19	RS-F	
7	31,3	**145**	8,23	23	RS-F	result converted °
8	38,0	176	11,9	33	RS-F	
16	33,0	153	9,00	25	RS-F	

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RA**	50-150 %	RA**	50-150 %
Number in RA	**4**	Anzahl im AB	**0**
Percent in RA	**40**	Prozent im AB	**0**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:
BF = MonoTrace ELISA, BioFront Technologies
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
40% (4) of the participants obtained a recovery rate by ELISA methods for the spiking level sample within the range of the AOAC-recommendation of 50-150%. For the spiked processed food matrix sample B, all recoveries were below the range of acceptance.

4.1.2 PCR Results: Lupin

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		
7	negative		positive		2/2 (100%)	ASU	
8	negative		positive		2/2 (100%)	ASU	
12	negative		positive		2/2 (100%)	ASU	
13	negative		positive	9,9	2/2 (100%)	ASU	result as lupin flour
16	negative		positive		2/2 (100%)	ASU	
9	negative	< 0,4	positive		2/2 (100%)	SFA-ID	
4	negative		negative		1/2 (50%)	div	
11	negative		positive		2/2 (100%)	div	
15	positive		negative		0/2 (0%)	div	

	Sample A		Sample B	
Number positive	1		7	
Number negative	8		2	
Percent positive	11		78	
Percent negative	89		22	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.
One positive result was obtained for sample A and two negative results were obtained for sample B by unknown methods or in-house methods.

Quantitative Valuation PCR: Sample B

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

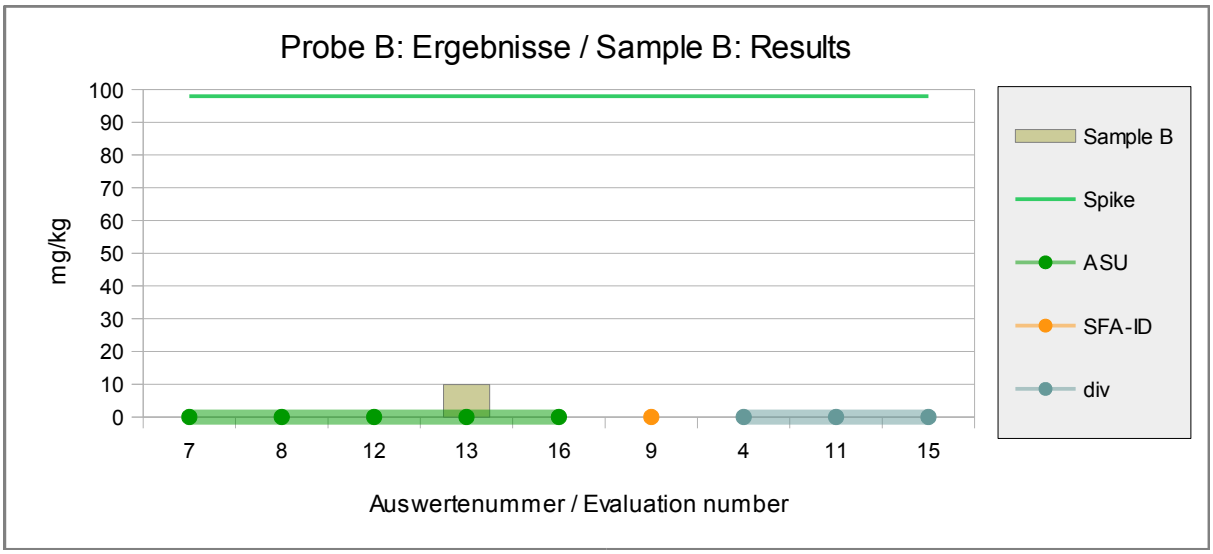

Abb./Fig. 10: PCR Results Lupin
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation PCR: Spiking Level Sample

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

Evaluation number	Lupin	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
7	positive			ASU	
8	positive			ASU	
12	positive			ASU	
13	positive	28,6		ASU	result as lupin flour
16	positive			ASU	
9	positive			SFA-ID	
4	positive			div	
11	positive			div	
15	positive			div	

Number positive	9
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:
ASU = ASU §64 Methode/method
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

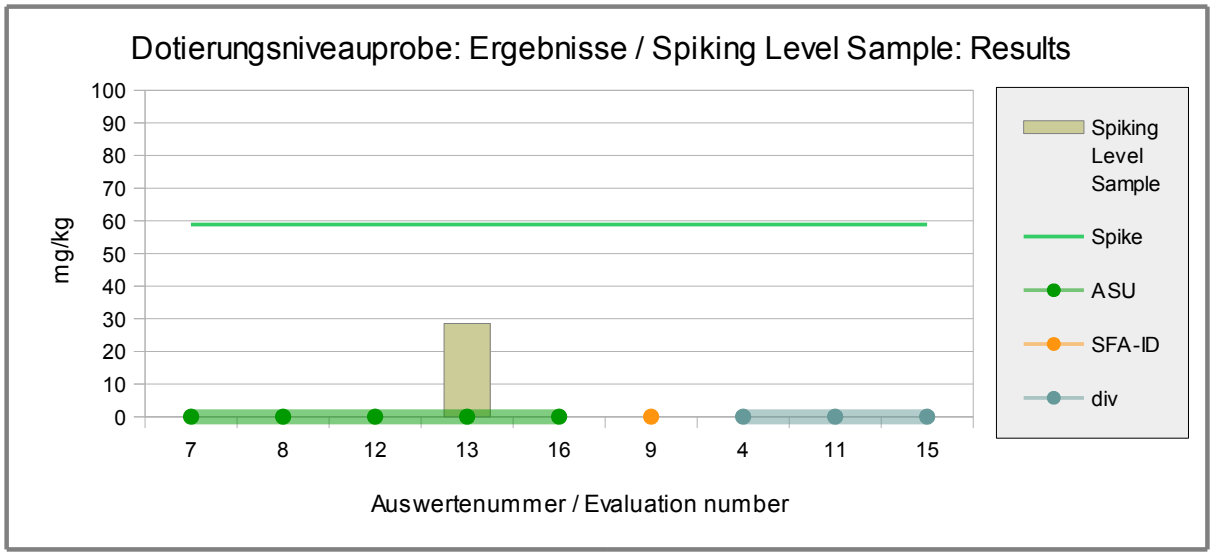

Abb./Fig. 11: PCR Results Lupin
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Lupin:
Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
7					ASU	
8					ASU	
12					ASU	
13	28,6	49	9,9	10	ASU	result as lupin flour
16					ASU	
9					SFA-ID	
4					div	
11					div	
15					div	

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

Methods:
ASU = ASU §64 Methode/method
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:
One participant determined quantitative results by PCR. For the spiking level sample, the recovery rate was 49%, just below the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B, the recovery rate was clearly lower.

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		
5	negative	0	positive	49,8	2/2 (100%)	BF	
12a	negative	<3,1	positive	72,0	2/2 (100%)	EF-R5	
2	positive	<4,0	positive	51,6	1/2 (50%)	IL	
14	negative	<4,0	positive	30,0	2/2 (100%)	IL	result converted °
1	negative	<6,6	positive	49,7	2/2 (100%)	RS	
6	negative	<5,0	positive	50,4	2/2 (100%)	RS	
7	negative	< BG	positive	49,7	2/2 (100%)	RS	
8	negative	<5,0	positive	51,0	2/2 (100%)	RS	
10	negative	<5,0	positive	29,4	2/2 (100%)	RS	
13	negative		positive	30,7	2/2 (100%)	RS	
12b	negative	<5,0	positive	55,0	2/2 (100%)	RS	
16	negative		positive	43,0	2/2 (100%)	RS	
11	negative	<10	positive	60,0	2/2 (100%)	RS-F	
15	negative		positive	40,0	2/2 (100%)	VT-R5	

° calculation p. 19

	Sample A		Sample B	
Number positive	1		14	
Number negative	13		0	
Percent positive	7		100	
Percent negative	93		0	
Consensus value	negative		positive	

Methods:

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

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.
One positive result for sample A was obtained below the limit of quantification.

Quantitative valuation of results: Sample B

Evaluation number	Sample B	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
5	49,8	0,26		BF	
12a	72,0	2,2		EF-R5	
2	51,6	0,41		IL	
14	30,0	-1,4		IL	result converted °
1	49,7	0,25	0,00	RS	
6	50,4	0,31	0,06	RS	
7	49,7	0,24	0,00	RS	
8	51,0	0,36	0,10	RS	
10	29,4	-1,5	-1,6	RS	
13	30,7	-1,4	-1,5	RS	
12b	55,0	0,70	0,43	RS	
16	43,0	-0,32	-0,54	RS	
11	60,0	1,1		RS-F	
15	40,0	-0,58		VT-R5	

° calculation p. 19

Methods:

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

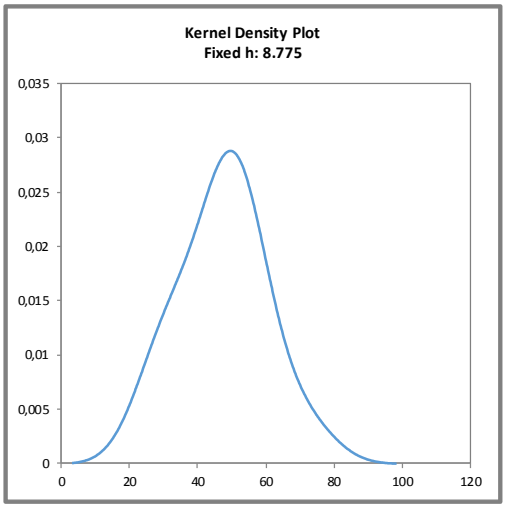

Abb. / Fig. 12:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a small shoulder at approx. 30 mg/kg.

Characteristics: Quantitative evaluation ELISA Gluten

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	14	8
Number of outliers	0	0
Mean	47,3	44,9
Median (X_{pt})⁺	49,8	**44,9**
Robust Mean (X_{pt})⁺⁺	**46,8**	49,7
Robust standard deviation (S*)	**12,4**	**11,0**
Target range:		
Target standard deviation σ_{pt}	**11,7**	**12,4**
lower limit of target range	**23,4**	**24,8**
upper limit of target range	**70,2**	**74,5**
Quotient S/σ_{pt}*	*1,1*	*0,89*
Standard uncertainty $U_{(Xpt)}$	*4,16*	*4,87*
Results in the target range	*13*	*8*
Percent in the target range	*93*	*100*

⁺ Assigned value (X_{pt}) for method RS: Median

⁺⁺ Assigned value (Xpt) for all results: Robust Mean

Methods:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

For method RS the median was used as the assigned value (see table above, see 3.1).

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 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 Xpt (robust mean / median) of the evaluations were 93% and 89% of the spiking level of gluten to sample B and thus within the recommendations for the applied methods (s. 3.4.3 and "recovery rates for gluten", p.44).

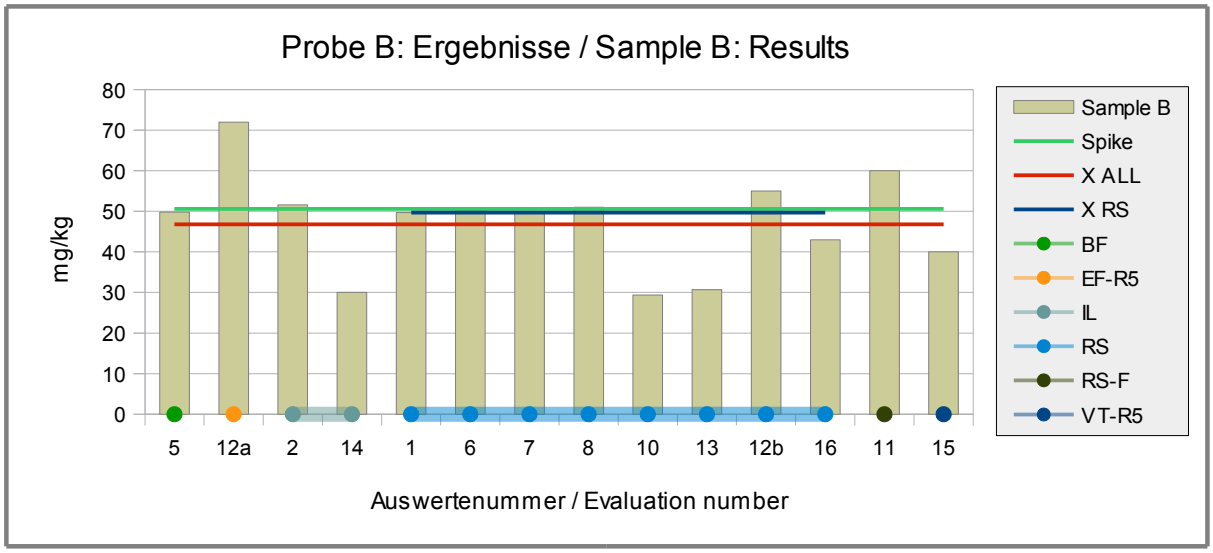

Abb./Fig. 13: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

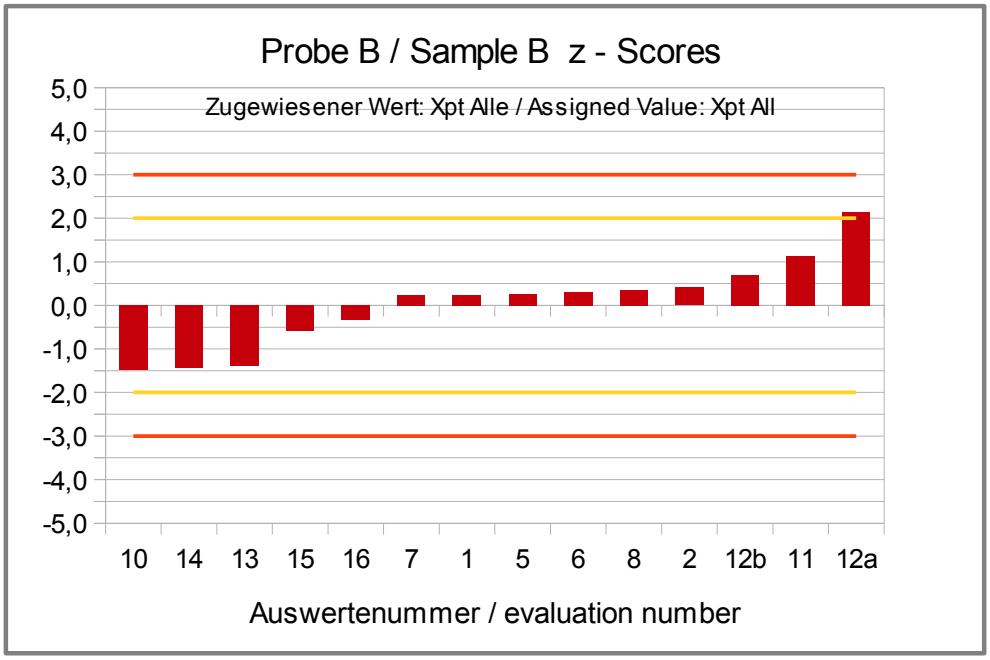

Abb./Fig. 14:
z-Scores (ELISA Results Gluten) Assigned value robust mean of all results

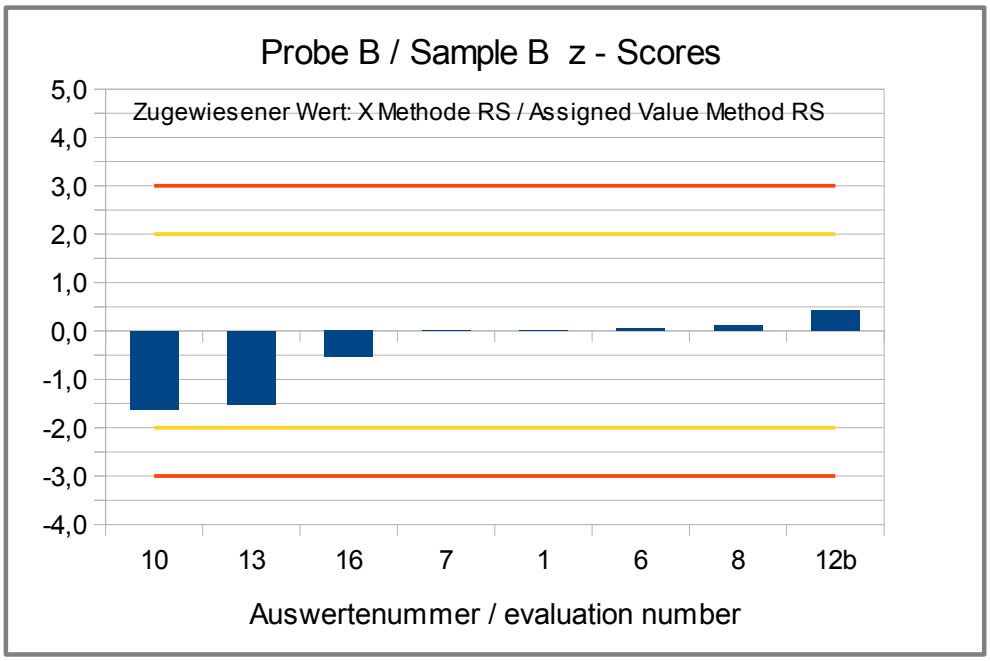

Abb./Fig. 15:
z-Scores (ELISA Results Gluten) Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Quantitative valuation of ELISA results: Spiking level sample

Evaluation number	Spiking Level Sample	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
5	50,1	0,6		BF	
12a	54,0	1,0		EF-R5	
2	140	8,9		IL	outlier X_{All}, excluded
14	110,0	6,1		IL	result converted °, outlier X_{All}, excluded
1	49,5	0,6	0,7	RS	
6	44,1	0,1	0,2	RS	
7	38,0	-0,5	-0,4	RS	
8	48,0	0,4	0,5	RS	
10	46,7	0,3	0,4	RS	
13	31,4	-1,1	-1,0	RS	
12b	45,0	0,1	0,2	RS	
16	36,0	-0,7	-0,6	RS	
11	40,0	-0,3		RS-F	
15	37,0	-0,6		VT-R5	

° calculation p. 19

Methods:

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

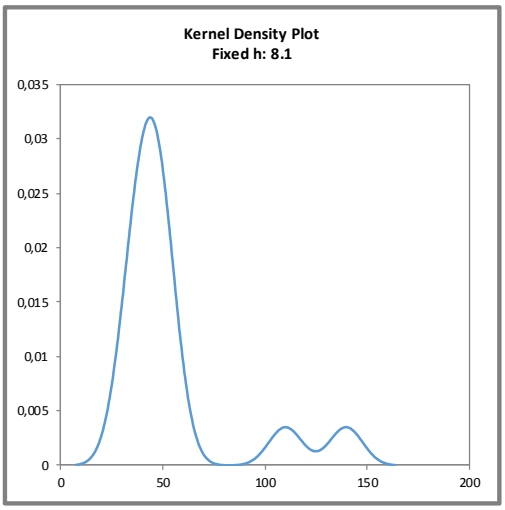

Abb. / Fig. 16:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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 two small side peaks at > 100 mg/kg (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 **	12	8
Number of outliers	2	-
Mean	43,3	42,3
Median	44,5	44,5
Robust Mean (X_{pt})	**43,4**	**42,4**
Robust standard deviation (S*)	**7,62**	**7,31**
Target range:		
Target standard deviation σ_{pt}	**10,8**	**10,6**
lower limit of target range	**21,7**	**21,2**
upper limit of target range	**65,1**	**63,6**
Quotient S/σ_{pt}*	*0,70*	*0,70*
Standard uncertainty $U_{(Xpt)}$	*2,75*	*3,23*
Results in the target range	*12*	*8*
Percent in the target range	*100*	*100*

** without results no. 2 and 14 (excluded)*

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 low variability of results. The quotients S/σ_{pt} were below 1,0. 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 143% and 140% 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.44).

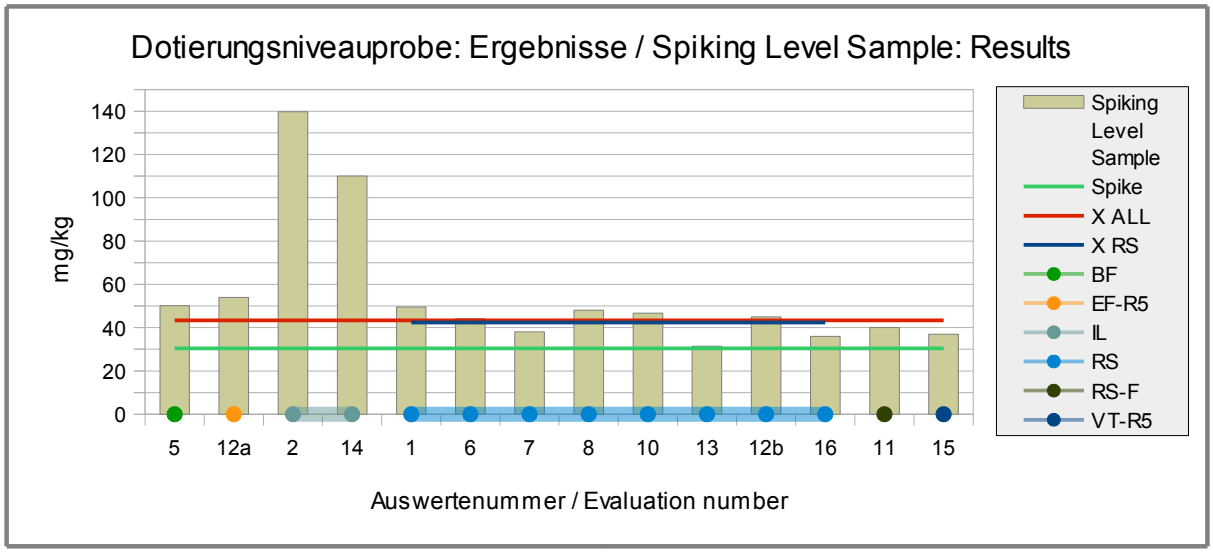

Abb./Fig. 17: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

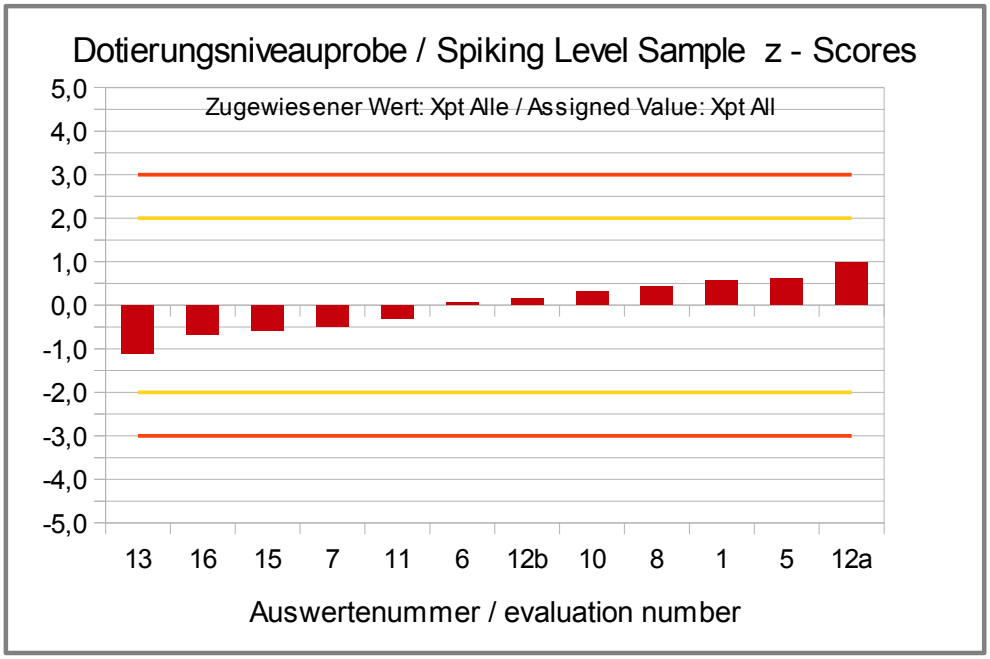

Abb./Fig. 18:
z-Scores (ELISA Results Gluten) Assigned value robust mean of all results

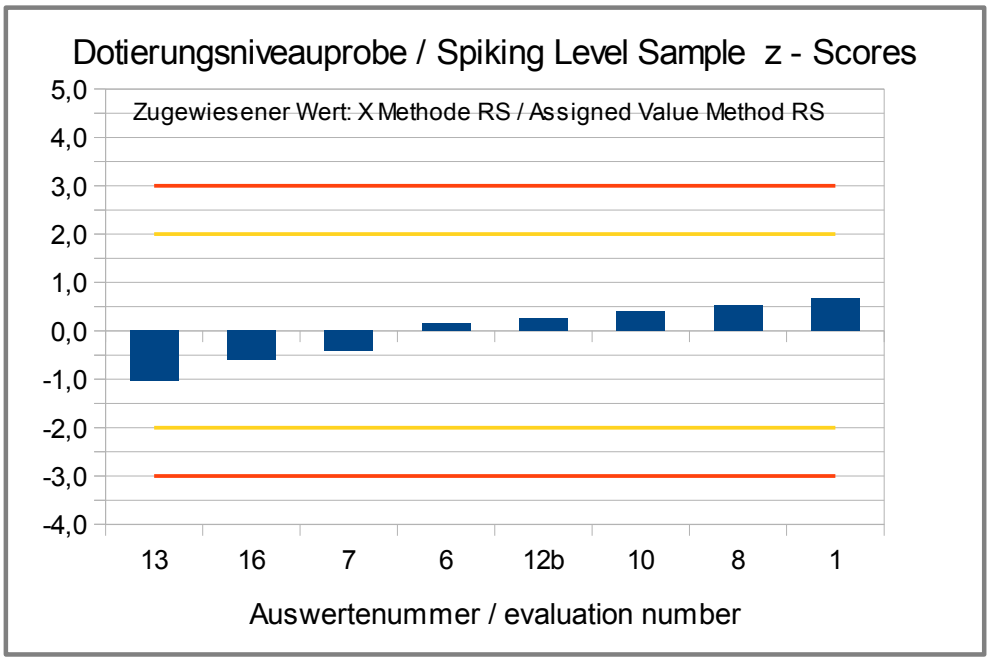

Abb./Fig. 19:
z-Scores (ELISA Results Gluten) Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Recovery Rates for Gluten:
Spiking level sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
5	50,1	165	49,8	**98**	BF	
12a	54,0	178	72,0	**142**	EF-R5	
2	140	460	51,6	**102**	IL	
14	110	362	30,0	**59**	IL	result converted °
1	49,5	163	49,7	**98**	RS	
6	44,1	**145**	50,4	**100**	RS	
7	38,0	**125**	49,7	**98**	RS	
8	48,0	158	51,0	**101**	RS	
10	46,7	154	29,4	**58**	RS	
13	31,4	**103**	30,7	**61**	RS	
12b	45,0	**148**	55,0	**109**	RS	
16	36,0	**118**	43,0	**85**	RS	
11	40,0	**132**	60,0	**119**	RS-F	
15	37,0	**122**	40,0	**79**	VT-R5	

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**7**	Number in RA	**14**
Percent in RA	**50**	Percent in RA	**100**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BF = MonoTrace ELISA, BioFront Technologies

EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins

IL = Immunolab

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

VT-R5 = Veratox, Neogen

Comments:
For the spiking level sample 50% (7) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 100% (14) of the obtained recovery rates were within the recommended range.

4.2.2 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		
13	negative		positive	150	2/2 (100%)	ASU	result as w heat
8	negative		positive		2/2 (100%)	div	
15	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		3	
Number negative	3		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

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

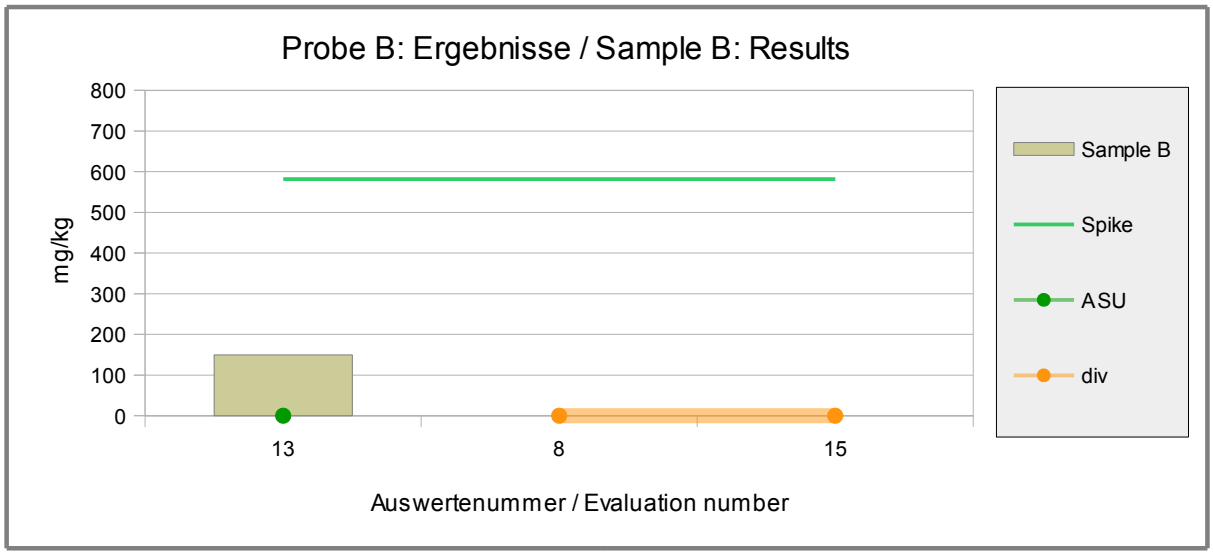

Abb./Fig. 20: PCR Results Wheat
green line = Spiking level
round symbols = Applied methods (see legend)

(Quantitative) Valuation PCR: Spiking Level Sample

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

Evaluation number	Wheat	Wheat	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
13	positive	321		ASU	result as wheat
8	positive			div	
15	positive			div	

Number positivee	3
Number negativee	0
Percent positivee	100
Percent negativee	0
Consensus value	positive

Methods:
ASU = ASU §64 Methode/method
div = not indicated / other method

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

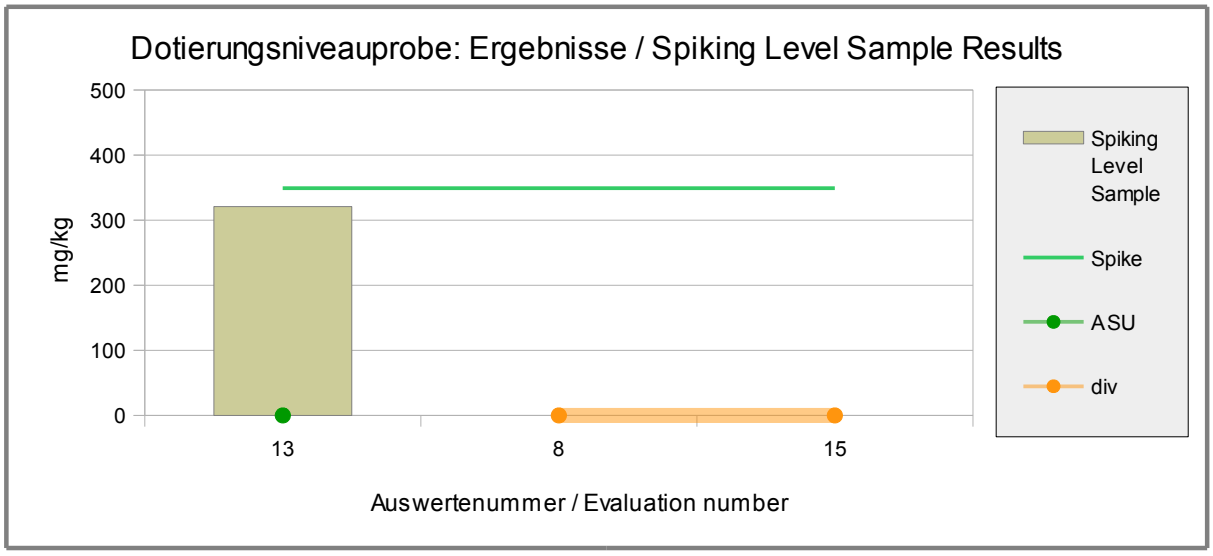

Abb./Fig. 21: PCR Results Wheat
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Gluten-containing Cereals (Wheat): Spiking Material Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
13	321	**92**	150	26	ASU	result as wheat
8					div	
15					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**1**	Anzahl im AB	**0**
Percent in RA	**100**	Prozent im AB	**0**

Methods:

ASU = ASU §64 Methode/method

div = not indicated / other method

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

** Range of acceptance of AOAC for allergen ELISAS

Comments:

One participant determined quantitative results by PCR. For the spiking level sample, the recovery rate was within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B, the recovery rate was clearly lower.

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: Lupin

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	PCR Test-Kit + Manufacturer
BF	5	04.04.19	negative	0	positive	6,3	positive	41,7	0,13	1		Lupin flour	MonoTrace Lupin ELISA kit, BioFront Technologies
EF	12	12.3.	negative	< 2	positive	12	positive	130	1,5	2		Lupin	SensiSpec ELISA Lupin, Eurofins
EF	14	04.03.19	negative	< 2	positive	16	positive	90	0.2	2		Lupin	SensiSpec ELISA Lupin, Eurofins
IL	1	10.04.19	negative		positive	14,79	positive	109,15	0,3	2		Lupin	Immunolab Lupin ELISA
IL	2	05.03.19	positive	< 2	positive	10,7	positive	69,8	0,3	2		Lupin	Immunolab Lupin ELISA
RS	9		negative	< 1	positive	8	>27			1		Lupin protein	Ridascreen Lupin R-Biopharm
RS-F	3	07.03.	positive	2,4	positive	9,5	positive	18,1	0,7	1	11,5	Lupin protein	Ridascreen FAST Lupin R6102
RS-F	6	10.03.19	negative	< 1	positive	6,75	positive	34,86	1	1	31,94	Lupin protein	
RS-F	7	20.03.19		2,75		22,48		85,49		2,5		Food (Lupin)	RIDASCREEN® Fast Lupin R6102, r-biopharm
RS-F	8	11.03.19	positive	1,9	positive	11,9	positive	38	0,7	1	20	Protein	Fast Lupin; r-biopharm
RS-F	16	06.03.19	positive	1,3	positive	9	positive	33	1	1	47	Lupin protein	

* 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	
BF	5	Monoclonal antibody based assay	1:20 extraction for 10 minutes @ 60C	NO	
EF	12	detects lupin proteins	As Per Kit Instructions	yes	HU0030011:2
EF	14				
IL	1			no	no further dillution needed
IL	2			yes	
RS	9			no	
RS-F	3	Lupinus albus, L. luteus, L. angustifolius	As Per Kit Instructions	yes	
RS-F	6	As Per Kit Instructions	As Per Kit Instructions	Yes	R-Biopharm FAST Lupin R6102
RS-F	7	Antibody detects specifically all lupin proteins, including gamma-Conglutin as w ell as Lupinus albus, luteus and angustifolius.	As Per Kit Instructions	yes	
RS-F	8	g-Conglutin	Allergen Extraction buffer/10min/60°C	yes	
RS-F	16		RIDASCREEN FAST Lupine	yes	

5.1.2 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 / food protein	PCR Test-Kit + Manufacturer
BF	5	04.04.19	negative	0	positive	49,8	positive	50,1	0,36	2		Please select!	MonoTrace Gluten ELISA kit, BioFront Technologies
EF-R5	12a	7.3.	negative	< 3,12	positive	72	positive	54	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins
IL	2	05.03.19	positive	<4	positive	51,6	positive	139,7	0,6	4		Gluten	Immunolab Gliadin/Gluten ELISA
IL	14	04.03.19	negative	< 2	positive	15	positive	55	0.3	2		Gliadin	Immunolab Gliadin/Gluten ELISA
RS	1	16.04.19	negative	< 6,6	positive	49,7	positive	49,5	2,5	6,6		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	6	08.03.19	negative	<5	positive	50,4	positive	44,08	5	5	23,59	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	7	26/03/19 27/03/19 09/04/19	-	< BG	-	49,66	-	38,02		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8	14.03.19	negative	< 5,00	positive	51	positive	48	1	5	20	Protein	Ridasreen Gliadin; r-biopharm
RS	10	05.04.19	negative	< 5.0	positive	29,4	positive	46,7	5	5	20	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	13	13.03.19	negative		positive	30,7	positive	31,4	2	5	50	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	12b	6.3.	negative	< 5	positive	55	positive	45	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	16	05.03.19	negative		positive	43	positive	36	5	5	43	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS-F	11		negative	<10	positive	60	positive	40	1	10		Gluten	Ridascreen® FAST Gliadin R7002, R-Biopharm
VT-R5	15	11.04.19	negative		positive	40	positive	37	5	5		Gluten	Veratox Gliadin R5, 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	
BF	5	Monoclonal antibody based assay	1:40 extraction ratio for 1 hour at 60C	NO	
EF-R5	12a	R5 Mendez, detects prolamins from wheat, rye and oat	As Per Kit Instructions	yes	
IL	2			yes	
IL	14				
RS	1			yes	no further dillution needed
RS	6	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS	7	Monoclonal R 5 Antibody	As Per Kit Instructions	yes	
RS	8	R5; Prolamins	Coctail solution/40 min/50°C/ 60% EtOH/60 min/RT	yes	
RS	10			yes	
RS	13	Gliadin	Cocktail extraction solution	yes	1g sample weight
RS	12b	R5 Mendez, detects prolamins from wheat, rye and oat	As Per Kit Instructions	yes	
RS	16	R5 Antibody	Extraction with milk powder for sample A and B	yes	
RS-F	11			yes	
VT-R5	15			yes	

5.1.3 PCR: Lupin

Meth. Abr.	Evaluation number	Date of analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food / food protein	PCR Test-Kit + Manufacturer
ASU	7	21.03.19	negative		positive		positive					Lupin-DNA	ASU §64 Methode/method
ASU	8	10.04.19	negative		positive		positive		0,005 %			Lupin-DNA in Maize-DNA	Hausmethode (entspr. ASU §64 LFGB L 08.00-58(V))
ASU	12	6.3.	negative		positive		positive		0,5			Lupin-DNA	ASU §64 Methode/method
ASU	13	13.03.19	negative		positive	9,9	positive	28,6	5	10	50	Lupin flour	ASU §64 Methode/method
ASU	16	25.03.19	negative		positive		positive					Lupin-DNA	ASU §64 Methode/method
SFA-ID	9		negative	< 0,4	positive		positive		0,4			Lupin-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	4	21.03.19	negative		negative		positive		100				In House Method
div	11		negative		positive		positive						In House AllAllC, rt-PCR, Demmel et al.
div	15	16.04.19	positive		negative		positive		10			Lupin-DNA	

* 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
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	7	ITS 1 of nuclear rDNA	Extraction: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
ASU	8	Lupin spp.	CTAB-Extraction / Proteinase K / Dneasy Mericon Food Kit (Qiagen) / RT-PCR (45 cycles)	yes	
ASU	12		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-rime PCR 45 cycles	yes	§ 64 LFGB L 08.00-58 (V):2011-06
ASU	13	Internal transcribed Spacer 1 (ITS-1) Gene (multicopy-Sequence) (129bp)	Extraction: CTAB-Precipitation method, s. e.g. ASU L 18.00-22 determination: ASU L 08.00-59 : 2013-01	yes	calibration/quantification by Matrix Standards, spiked Material: Lupin flour (Lupinus albus)
ASU	16	ITS-1 (multicopy-Sequenz)	Extraktion mit Machery & Nagel NucleoSpin Food Kit	yes	
SFA-ID	9			no	
div	4	L1PR10.1A(Ypro10.1a) gene	Phenol/Chloroform Extraction afterw ards Dneasy Plant mini kit, End point PCR, 45 Cycles, PAGE	yes	
div	11	ITS	CTAB-Wizard, rt-PCR / 45 Cycles	yes	
div	15			yes	

5.1.4 PCR: Wheat (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 / food protein	PCR Test-Kit + Manufacturer
ASU	13	20.03.19	negative		positive	150	positive	321	50	100	50	Wheat	
div	8	10.04.19	negative		positive		positive		0,001 %			Wheat-DNA in Maize-DNA	in-house method
div	15	16.04.19	negative		positive		positive		10			Wheat-DNA	

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	13	Lipid-Transferprotein- Gene (ltp) (61 bp)	Extraction: CTAB-Precipitation method, s. e.g. ASU L 18.00-22 determination: ASU L 08.00-59 : 2013-02	yes	calibration/quantification by Matrix Standards, spiked Material: Wheat flour
div	8	w-gliadin (Wheat)	CTAB-Extraction / Proteinase K / Dneasy Mericon Food Kit(Qiagen) / RT-PCR (45 Cycles)	yes	
div	15			yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 02-2019 Sample B

Weight whole sample	1,75	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	44,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	3,46	84	48,6
2	5,05	115	45,5
3	5,00	122	48,8
4	5,04	117	46,4
5	5,02	130	51,8
6	4,99	131	52,5
7	5,00	115	46,0
8	4,99	128	51,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	117,7	Particles
Standard deviation	6,63	Particles
χ^2 (CHI-Quadrat)	2,61	
Probability	**92**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	48,9	mg/kg
Standard deviation	2,75	mg/kg
rel. Standard deviaton	5,6	%
Horwitz standard deviation	8,9	%
HorRat-value	**0,63**	
Recovery rate	110	%

Microtracer Homogeneity Test
DLA 02-2019 Spiking Level Sample

Weight whole sample	1,52	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	14,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	54	21,6
2	4,97	40	16,1
3	5,01	39	15,6
4	5,09	43	16,9
5	5,12	50	19,5
6	5,03	39	15,5
7	5,03	47	18,7
8	5,14	51	19,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,4	Particles
Standard deviation	5,76	Particles
χ^2 (CHI-Quadrat)	5,12	
Probability	**65**	%
Recovery rate	121	%

Normal distribution		
Number of samples	8	
Mean	18,0	mg/kg
Standard deviation	2,28	mg/kg
rel. Standard deviaton	12,7	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,2**	
Recovery rate	121	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 02-2019***
PT name	***Allergens II: Lupin and Wheat in „gluten-free“ Bread***
Sample matrix (processing)	***Samples A + B:*** *"Gluten-free" Bread (baked at 190°C) / ingredients: Bake Mix (Cornstarch, linseed flour 12%, buckwheat flour 8%, pea bran, rice bran, vegetable fiber (psyllium), sugar, thickener: guar gum, salt), water, oil, baker's yeast, salt other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Lupin (Lupin protein, DNA), Wheat (gluten, DNA)* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably, the total sample amount is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/kg*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest April 12th 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SWITZERLAND
		Germany
		USA
		SWITZERLAND
		ITALY
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN
		Germany
		GREAT BRITAIN
		Germany
		GREECE

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

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

7. Index of references

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