Evaluation Report
proficiency test

ptAL02 (2020)

Allergens II:

Soya and Wheat (Gluten)

in “gluten free” Pastry (Cookies)

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

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

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

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	ptAL02 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (8. Juni 2020) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 8. Juni 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Content

1. Introduction

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 are common in commerce gluten-free cookies. 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:
An additional ingredient were cookies baked (150°C, 40 min) with the spiking material containing the allergenic ingredients soya and wheat (mesh <500 µm). After 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 in up to 4 additional steps and homogenized in each case until the total quantity had been reached.
The **spiking level sample** was produced with the allergenic compounds above mentioned by multi-stage addition of potato powder (mesh 500 µm) and homogenization.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spikin Level Sample**
Rice-Cocoa Cookies, gluten free Ingredients: Cane sugar, rice flour, corn starch, eggs, corn flour, rice starch, sunflower oil, skimmed cocoa powder, shea butter, apple fiber, salt, raising agent: potassium tartrate, sodium carbonate, ammonium carbonate, thickener: guar gum, cocoa extract, natural flavor, antioxidant: rosemary extracts Nutrients per 100 g: Fat 12 g, Carbohydrates 74 g, Protein 4,8 g, Salt 0,6 g	62,1 g/100g	62,5 g/100g	-
Butter Cookies, gluten free Ingredients: Corn starch, corn flour, sugar, sunflower oil, pure butter fat, chicken egg, invert sugar syrup, dry milk product, lowfat cocoa powder, thickener: xanthan, salt, flavors, raising agent: sodium carbonates, ammonium carbonates, acidifying agents: citric acid Nutrients per 100 g: Fat 15 g, Carbohydrates 78 g, Protein 2,6 g	37,2 g/100 g	37,5 g/100g	-
Cookies, baked 150°C, 40 min Ingredients: Sugar, corn starch, corn flour, rice flour, lentil flour, butter, eggs, modified tapioca starch, thickener: locust bean gum, salt and allergens Food soya flour and wheat flour (see below)	0,689 g/100g	–	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	–	–	99,9 g/100 g
Soya: - as soya flour, untoasted* - thereof 33,8% total protein** - thereof soy trypsin inhibitor***	 65,9 mg/kg° 22,3 mg/kg° 3,35 mg/kg°	–	 69,6 mg/kg 23,5 mg/kg 3,53 mg/kg
Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) - as wheat flour* - thereof 10,1% total protein** - thereof gluten***	 208 mg/kg° 21,0 mg/kg° 18,1 mg/kg°	–	 409 mg/kg 41,3 mg/kg 35,6 mg/kg
further Ingredients: Maltodextrin and silicon dioxide	<0,1 g/100 g	–	<0,1 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture

** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,71 for soya protein and F=5,7 for wheat protein)

*** Protein contents according to literature values (approx. 8,7% gluten in wheat flour [36-38]); approx. 15% soybean trypsin inhibitor in soy protein [39]

°Specified amounts of the allergenic ingredients are part of the baked cookies

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples A and the spiking level sample showed a probability of 90% and 100%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value 0,8 or 0,4. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample A

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

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample A by all ELISA tests for wheat protein/gluten/gliadin (Immunolab, Morinaga and AgraQuant G12) and soya (AgraQuant) (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

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

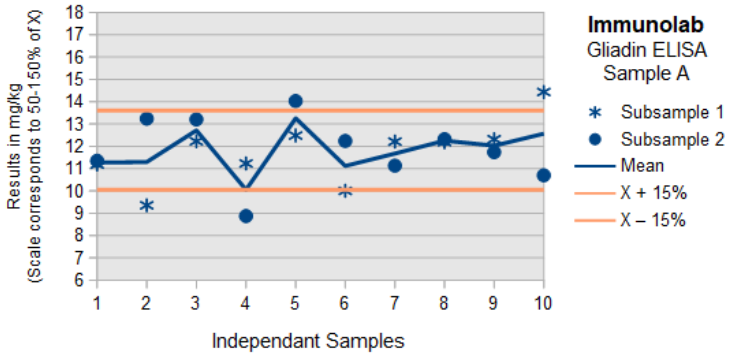
ELISA-Tests: Homogenität Weizenprotein / Homogeneity Wheat protein

Immunolab Gliadin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gliadin 11,8 ± 0,56 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	11,2	11,4	11,3
2	9,36	13,2	11,3
3	12,2	13,2	12,7
4	11,2	8,9	10,1
5	12,5	14,0	13,3
6	10,0	12,2	11,1
7	12,2	11,1	11,7
8	12,2	12,3	12,3
9	12,3	11,7	12,0
10	14,4	10,7	12,6

General average X	11,8	
SD of sample means Sx	0,93	7,9%
SD w ithin-samples Sw	1,05	8,9%
SD betw een-samples Ss	0,56	4,8%

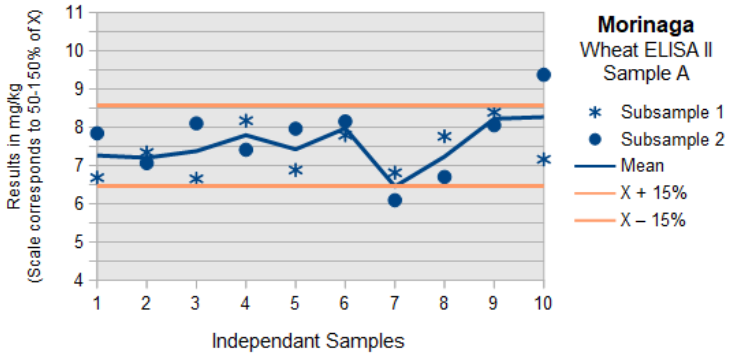


Morinaga Wheat/Gluten ELISA Kit II

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Wheat protein 7,02 ± 0,39 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	6,18	7,34	6,76
2	6,84	6,56	6,70
3	6,15	7,60	6,88
4	7,67	6,91	7,29
5	6,38	7,46	6,92
6	7,29	7,65	7,47
7	6,31	5,59	5,95
8	7,26	6,20	6,73
9	7,89	7,55	7,72
10	6,66	8,87	7,77

General average X	7,02	
SD of sample means Sx	0,55	7,9%
SD w ithin-samples Sw	0,55	7,8%
SD betw een-samples Ss	0,39	5,6%

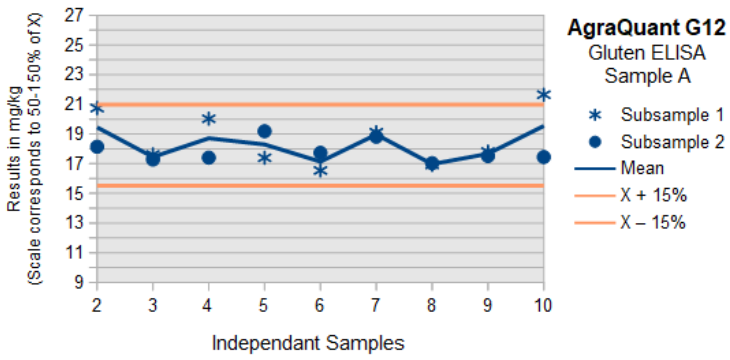


AgraQuant G12 ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 18,2 ± 0,68 mg/kg

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

General average X	18,2	
SD of sample means Sx	0,98	5,4%
SD w ithin-samples Sw	1,00	5,5%
SD betw een-samples Ss	0,68	3,7%



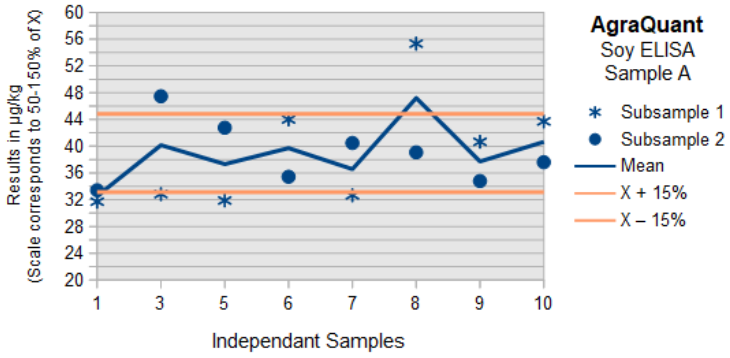
ELISA-Tests: Homogenität Soja / Homogeneity Soya

AgraQuant Soya ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Soya trypsin inhibitor 39,0 ± 2,3 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Mean µg/kg
1	31,7	33,4	32,6
3	32,9	47,5	40,2
5	31,9	42,8	37,3
6	44,0	35,4	39,7
7	32,7	40,5	36,6
8	55,3	39,1	47,2
9	40,7	34,8	37,7
10	43,7	37,6	40,7

General average X	39,0	
SD of sample means Sx	4,20	10,8%
SD within-samples Sw	5,01	12,9%
SD between-samples Ss	2,26	5,8%



2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the spiking level sample was approx. 0,33 (17°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 8th week of 2020. The testing method was optional. The tests should be finished at 4th May 2020 the latest (extended).

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

*There are two different samples A and B possibly containing the allergenic parameters **Soya and Wheat (Gluten)** in the range of mg/kg in the matrix of „**gluten-free**" **cookies**. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The **"spiking level sample"** contains the allergens in a simple matrix in **similar amounts** without further processing and should be analysed like a normal sample.*

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

2.3 Submission of results

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

24 of 25 participants submitted their results in time. One participant submitted no results.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [25, 26, 27, 28]. It is for this reason that we contrast the results of the present proficiency test with several assigned values.
Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.
For quantitative results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

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

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

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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 18 – 37% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

The Working Group on Prolamin Analysis and Toxicity (WGPAT) coordinated a collaborative study with two commercial ELISA test kits for the determination of gluten using the monoclonal R5 antibody [24]. 12 food samples with gliadin in the range of 0 - 168 mg/kg were analyzed by 20 laboratories. Recovery rates ranged between 65 and 110%, relative repeatability deviations ranged from 13 - 25% (method 1) and 11 - 22% (method 2) while the relative reproducibility standard deviations ranged from 23 - 47% (method 1) and 25 - 33% (method 2). According to the authors both ELISA test kits fulfilled therefore the current validation criteria for ELISA methods [24].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [27]. The mean values for two matrices were in the concentration range of 0,3 - 16,1 mg/kg and 1,2 - 20,4 mg/kg, respectively. The lowest relative reproducibility standard deviations of the five test kits were for dark chocolate in the range of 20 - 42% and for cookies in the range of 23 – 61%.

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-35]

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

3.4.3 Value by perception

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

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

Table 3: ELISA-Validation

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

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

Table 4: PCR-Validation

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

(a) = Trueness / Richtigkeit

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

3.5 z-Score

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

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

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

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

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

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

3.5.1 Warning and action signals

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

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

3.6 z'-Score

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

The calculation is performed by:

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

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

3.9 Figures of assigned values

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

3.10 Recovery rates: Spiking

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

4. Results

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

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

To ensure the **comparability of quantitative results** DLA harmonized participants' results giving different specifications (e.g. as protein or as allergenic food) as far as possible.
ELISA-Results given as **soy flour** were converted into total **soy protein** using the analysed protein content of soy flour (see page 5).
One ELISA result, which was reportet as **soy trypsin inhibitor (STI)** was first converted into soy flour using the test kit manufacturer's specifications (Immunolab: factor 42) and then converted into **soy protein** using the experimentally determined protein content of the soy flour.
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 (Xpt)	$\boldsymbol{Xpt_{ALL}}$	$\boldsymbol{Xpt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (Xpt)		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σpt *or* $\sigma pt'$		
lower limit of target range ($Xpt - 2\sigma pt$) or ($Xpt - 2\sigma pt'$)°		
upper limit of target range ($Xpt + 2\sigma pt$) or ($Xpt + 2\sigma pt'$)°		
Quotient S*/σpt *or* S*/$\sigma pt'$		
Standard uncertainty $U(Xpt)$		
Number of results in target range		
Percent in target range		

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

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

4.1 Proficiency Test Soya

4.1.1 ELISA Results: Soya (as soy protein)

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
6	negative	< LOQ	negative	< LOQ	1/2 (50%)	AQ	
8	positive	2,60	negative	<LOD	2/2 (100%)	AQ	
17	negative	0,0200	negative	-4,13	1/2 (50%)	AT	
23	positive	25,0	negative	<0,5	2/2 (100%)	IL-SP	
2	positive	0,341	negative	<0,57	2/2 (100%)	IL-STI	Result sample A <LOQ, Result converted°
20	positive	0,0500	negative	<0,04	2/2 (100%)	IL-STI	Result given as STI?
5	positive	16,0	negative	<0,31	2/2 (100%)	MI-II	
4	positive	17,0	negative	< BG	2/2 (100%)	RS-F	
7	positive	13,8	negative	<2,5	2/2 (100%)	RS-F	
9a	positive	10,4	negative	<2,5	2/2 (100%)	RS-F	
10	positive	4,39	negative	<0,85	2/2 (100%)	RS-F	Result converted °
12	positive	20,3	negative		2/2 (100%)	RS-F	
14	positive	16,4	negative		2/2 (100%)	RS-F	
19	positive	18,4	positive	5,70	1/2 (50%)	RS-F	
21	positive	13,4	negative	<2,5	2/2 (100%)	RS-F	
22a	positive	13,1	negative	<2,5	2/2 (100%)	RS-F	
9b	-		negative	<1,17	1/1 (100%)	VT	
13	positive	2,80	negative	0	2/2 (100%)	VT	Result given as soy flour?
22b	negative	<0,85	negative	<0,85	1/2 (50%)	VT	Result converted °
24	positive	2,53	negative	<2,5	2/2 (100%)	VT	Result given as soy flour?

° calculation see p. 19

	Sample A		Sample B	
Number positive	16		1	
Number negative	3		19	
Percent positive	84		5	
Percent negative	16		95	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
AT = AlerTox Sticks (Lateral Flow), Biomedal
IL-SP = Immunolab Soy Protein Total
IL-STI = Immunolab Soy Trypsin Inhibitor
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

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

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Soy Protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
6	< LOQ			AQ	
8	2,60			AQ	Result excluded
17	0,0200			AT	Result excluded
23	25,0	2,5		IL-SP	
2	0,341			IL-STI	Result < LOQ, Result converted°, Result excluded
20	0,0500			IL-STI	Result given as STI? Result converted °
5	16,0	0,15		MI-II	
4	17,0	0,41	0,69	RS-F	
7	13,8	-0,43	-0,21	RS-F	
9a	10,4	-1,3	-1,1	RS-F	
10	4,39	-2,9	-2,8	RS-F	Result converted °
12	20,3	1,3	1,6	RS-F	
14	16,4	0,25	0,52	RS-F	
19	18,4	0,77	1,1	RS-F	
21	13,4	-0,53	-0,31	RS-F	
22a	13,1	-0,60	-0,38	RS-F	
9b				VT	
13	2,80			VT	Result excluded
22b	<0,85			VT	Result converted °
24	2,53			VT	Result excluded

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
AT = AlerTox Sticks (Lateral Flow), Biomedal
IL-SP = Immunolab Soy Protein Total
IL-STI = Immunolab Soy Trypsin Inhibitor
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

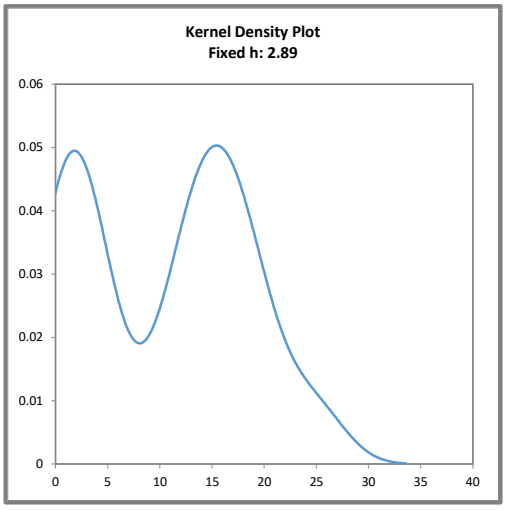


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a bimodal distribution of results with two maxima at approx. 2,5 mg/kg and approx. 16 mg/kg.

Characteristics: Quantitative evaluation ELISA soya (as soy protein)

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	11°	9
Number of outliers	6	0
Mean	15,3	14,1
Median	16,0	13,8
Robust Mean (X_{pt})	**15,4**	**14,5**
Robust standard deviation (S*)	**4,74**	**4,46**
Target range:		
Target standard deviation σ_{pt}	**3,85**	**3,63**
lower limit of target range	**7,71**	**7,25**
upper limit of target range	**23,1**	**21,8**
Quotient S/σ_{pt}*	*1,2*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*1,79*	*1,86*
Results in the target range	*9*	*8*
Percent in the target range	*82*	*89*

° *without results no. 2, 8, 13, 17, 20 and 24 (methods AQ, AT, IL and VT excluded in advance)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed method-dependent differences. Results of the methods, which were assigned to the maximum at approx. 16 mg/kg, were taken into account for the statistical evaluation. Due to partially implausible quantitative results, the methods of the lower maximum at approx. 2,5 mg/kg were not considered for a quantitative evaluation.

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

The robust means of the evaluations were 69% and 65% of the spiking level of soy protein to sample A and were thus in the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for soya").

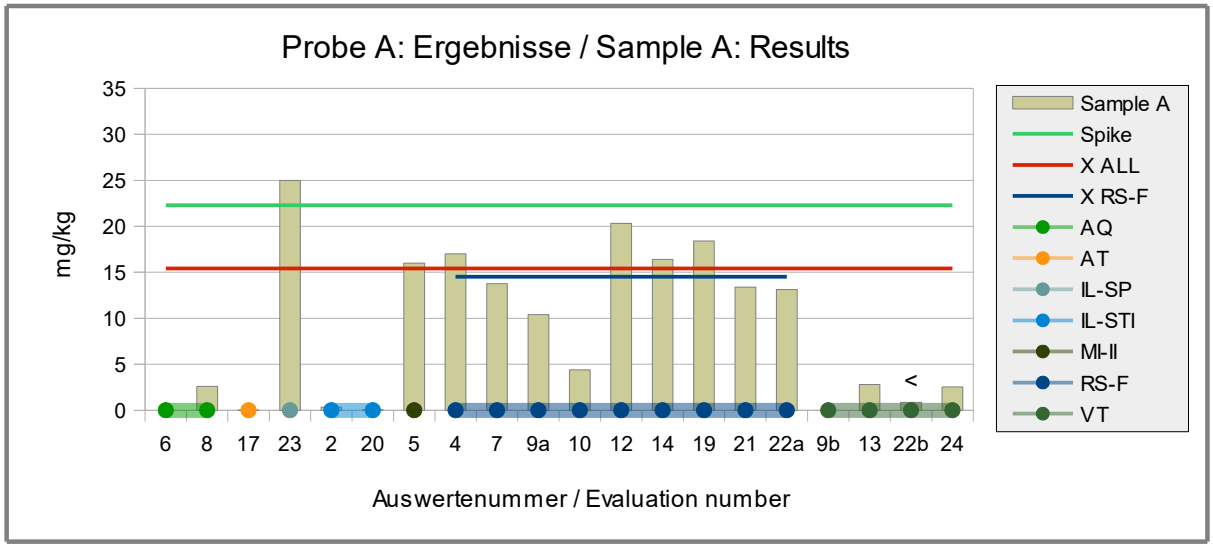


Abb./Fig. 2: ELISA Results soya (as soy protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

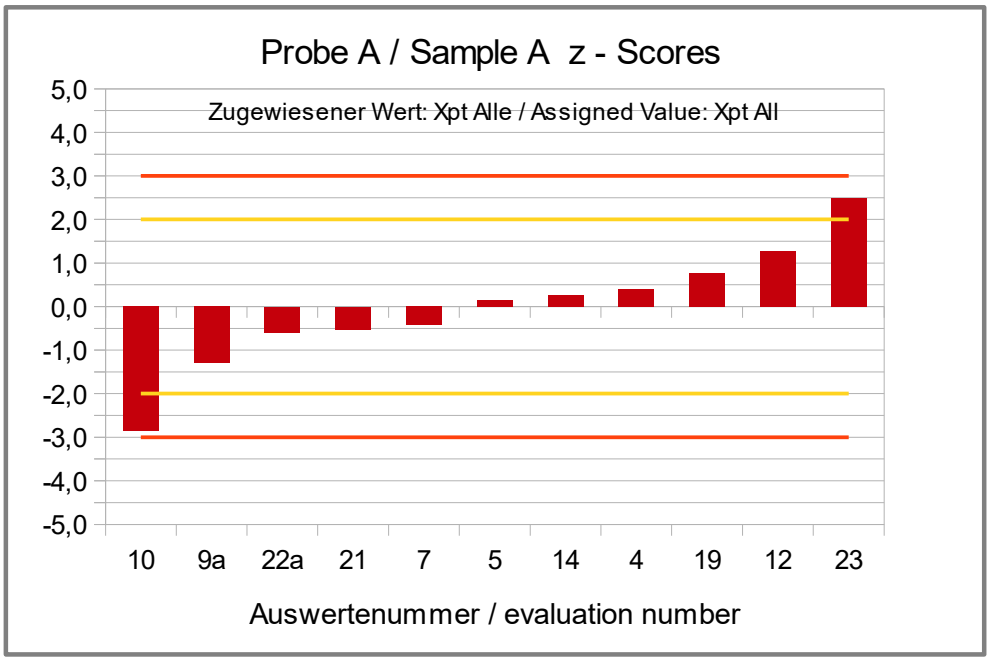


Abb./Fig. 3:
z-Scores ELISA Results soya (as soy protein)
Assigned value robust mean of all results

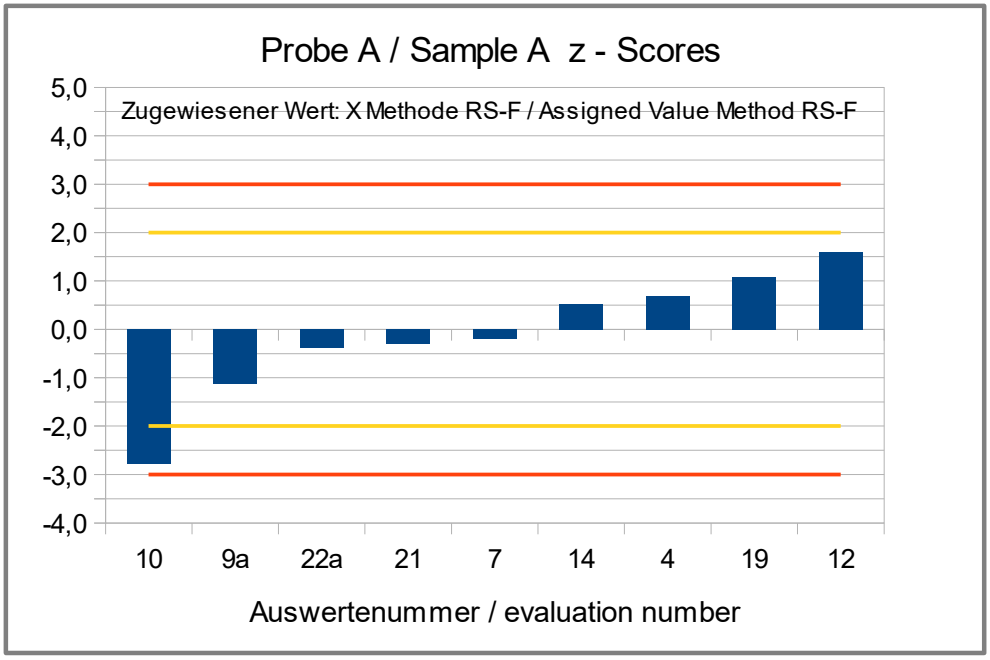


Abb./Fig. 4:
z-Scores ELISA Results soya (as soy protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Soy protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
6	60,0	2,3		AQ	
8	68,0	3,1		AQ	
17	124			AT	Outlier excluded
23	35,0	-0,32		IL-SP	
2	13,3	-2,6		IL-STI	Result converted °
20	1,54			IL-STI	Result given as STI? Outlier excluded
5	19,0	-2,0		MI-II	
4	32,0	-0,64	-0,32	RS-F	
7	33,2	-0,51	-0,19	RS-F	
9a	>20			RS-F	
10	5,41		-3,4	RS-F	Result converted°, Outlier Xpt_{ALL} excluded
12	39,7	0,17	0,56	RS-F	
14	39,0	0,10	0,48	RS-F	
19	37,1	-0,10	0,26	RS-F	
21	31,7	-0,67	-0,36	RS-F	
22a	38,6	0,06	0,44	RS-F	
9b	>11,8			VT	
13	66,0	2,9		VT	Result given as soy flour?
22b	27,4	-1,1		VT	Result converted °
24	>25			VT	Result given as soy flour?

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
AT = AlerTox Sticks (Lateral Flow), Biomedal
IL-SP = Immunolab Soy Protein Total
IL-STI = Immunolab Soy Trypsin Inhibitor
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

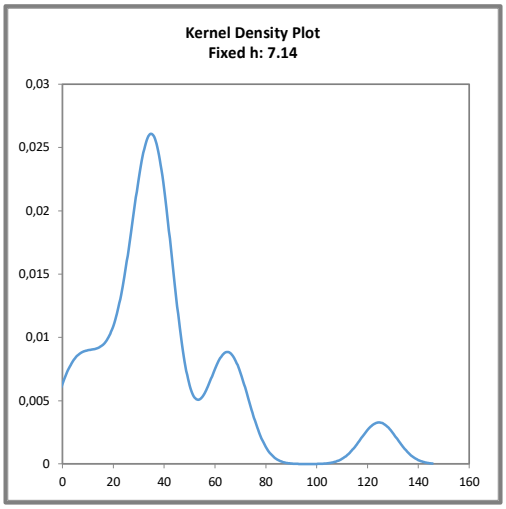


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetric distribution of results with a shoulder at approx. 10 mg/kg and two secondary peaks at approx. 65 mg/kg and 124 mg/kg, due to single results out of the target range.

Characteristics: Quantitative evaluation ELISA soya (as soy protein)

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	14°	8
Number of outliers	3	-
Mean	38,6	32,1
Median	36,1	35,2
Robust Mean (X_{pt})	**38,1**	**34,8**
Robust standard deviation (S*)	**17,2**	**5,04**
Target range:		
Target standard deviation σ_{pt}	**9,52**	**8,71**
lower limit of target range	**19,0**	**17,4**
upper limit of target range	**57,1**	**52,2**
Quotient S/σ_{pt}*	*1,8*	*0,58*
Standard uncertainty $U_{(Xpt)}$	*5,75*	*2,23*
Results in the target range	*9*	*7*
Percent in the target range	*64*	*88*

° *without results no. 2, 10 and 20 (excluded in advance)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no method-dependent differences.

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

The robust means of the evaluations were 162% and 148% of the spiking level of soy protein to the spiking level sample and were above or in the upper range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for soya").

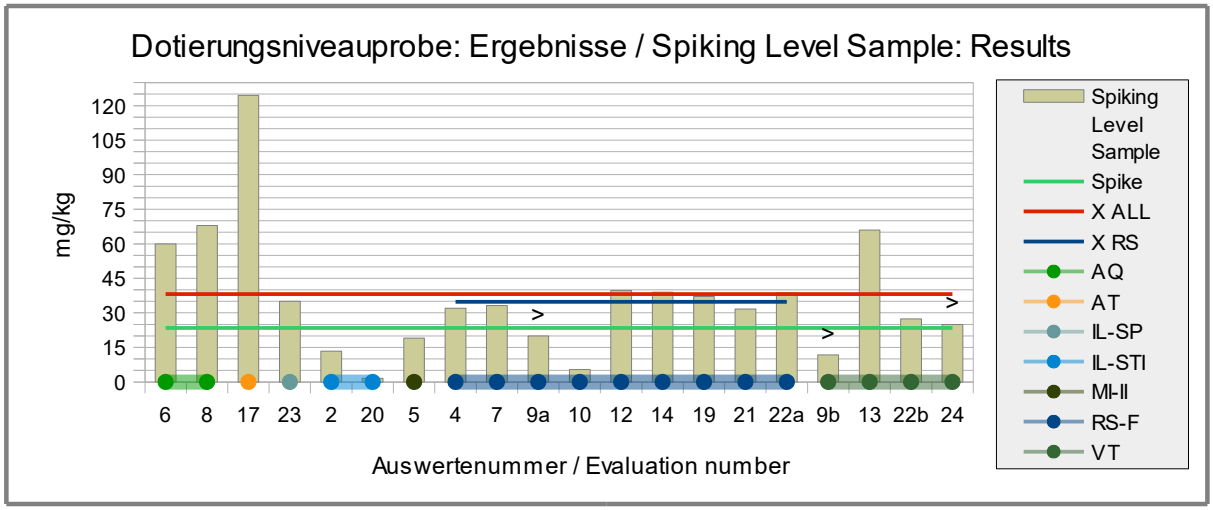


Abb./Fig. 6: ELISA Results soya (as soy protein)
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

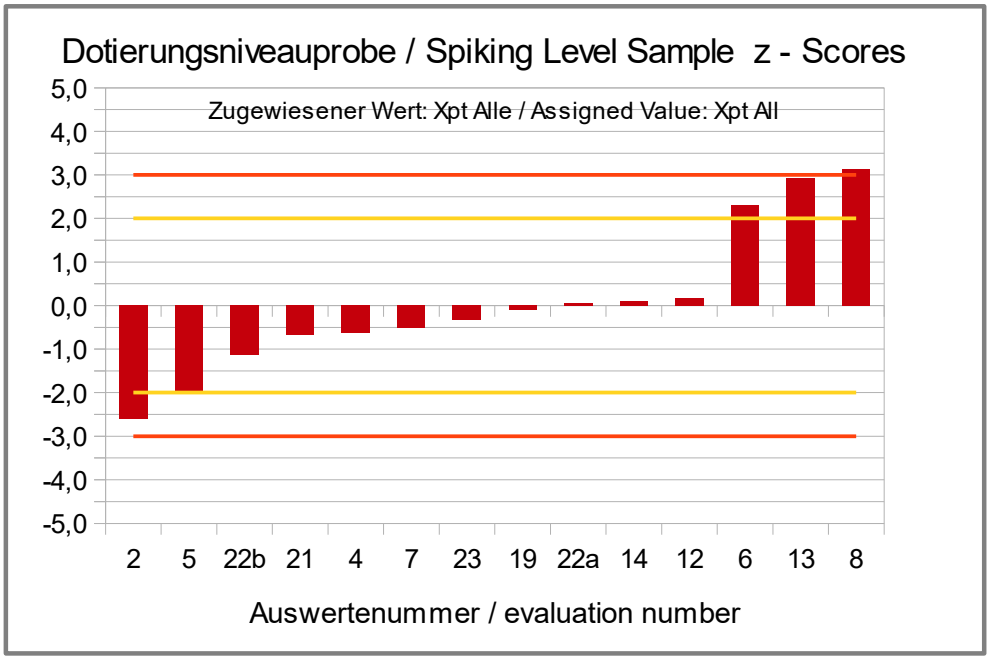


Abb./Fig. 7:
z-Scores ELISA Results soya (as soy protein)
Assigned value robust mean of all results

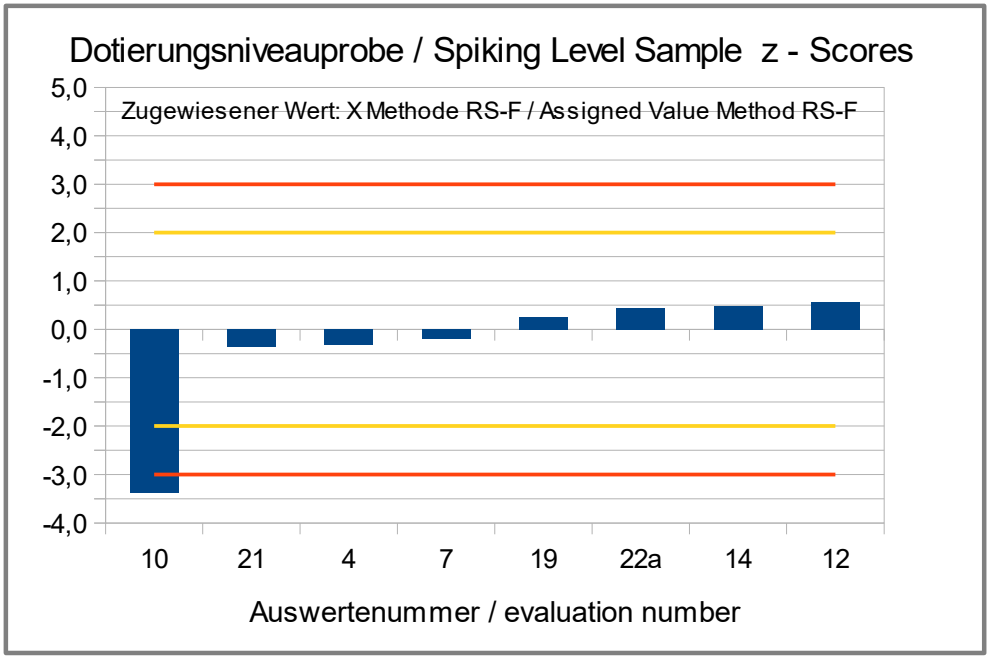


Abb./Fig. 8:
z-Scores ELISA Results soya (as soy protein) Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Recovery Rates with z-Scores ELISA for soya (as soy protein): Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
6	60,0	255	6,2	< LOQ			AQ	
8	68,0	289	7,6	2,60	12	-3,5	AQ	
17	124	530	17	0,0200	0	-4,0	AT	
23	35,0	**149**	2,0	25,0	**112**	0,48	IL-SP	
2	13,3	**57**	-1,7	0,341	2	-3,9	IL-STI	Result sample A <LOQ, Result converted °
20	1,54	7	-3,7	0,0500	0	-4,0	IL-STI	Result given as STI?
5	19,0	**81**	-0,77	16,0	**72**	-1,1	MI-II	
4	32,0	**136**	1,4	17,0	**76**	-0,95	RS-F	
7	33,2	**141**	1,7	13,8	**62**	-1,5	RS-F	
9a	>20			10,4	47	-2,1	RS-F	
10	5,41	23	-3,1	4,39	20	-3,2	RS-F	Result converted °
12	39,7	169	2,8	20,3	**91**	-0,36	RS-F	
14	39,0	166	2,6	16,4	**74**	-1,1	RS-F	
19	37,1	158	2,3	18,4	**83**	-0,70	RS-F	
21	31,7	**135**	1,4	13,4	**60**	-1,6	RS-F	
22a	38,6	164	2,6	13,1	**59**	-1,6	RS-F	
9b	>11,8						VT	
13	66,0	281	7,2	2,80	13	-3,5	VT	Result given as soy flour?
22b	27,4	**117**	0,66	<0,85		-4,0	VT	Result converted °
24	>25			2,53	11	-3,5	VT	Result given as soy flour?

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	7	Number in RA	9
Percent in RA	41	Percent in RA	53

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
AT = AlerTox Sticks (Lateral Flow), Biomedal
IL-SP = Immunolab Soy Protein Total
IL-STI = Immunolab Soy Trypsin Inhibitor
MI-II = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comment:

41% (7) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A 53% (9) of the obtained recovery rates were within the recommended range.
The related z-scores are based on the target standard deviation of 25%.

4.1.2 PCR Results: soya

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
4	positive		negative		2/2 (100%)	ASU	
7	positive		negative		2/2 (100%)	ASU	
11	positive	4,00	negative		2/2 (100%)	ASU	
12a	positive		negative		2/2 (100%)	ASU	
14	positive		negative		2/2 (100%)	ASU	
9	positive		negative		2/2 (100%)	SFA	
15	positive		positive		1/2 (50%)	SFA	
22	positive	49,6	negative	<1	2/2 (100%)	SFA-ID	Given as soya DNA
5	positive		negative		2/2 (100%)	div	
12b	positive		negative		2/2 (100%)	div	
16	positive	10,4	negative	<2,5	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	11		1	
Number negative	0		10	
Percent positive	100		9	
Percent negative	0		91	
Consensus value	positive		negative	

Methods:

ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

Quantitative valuation of PCR: Sample A

No quantitative valuation was done, because there were too few results available.

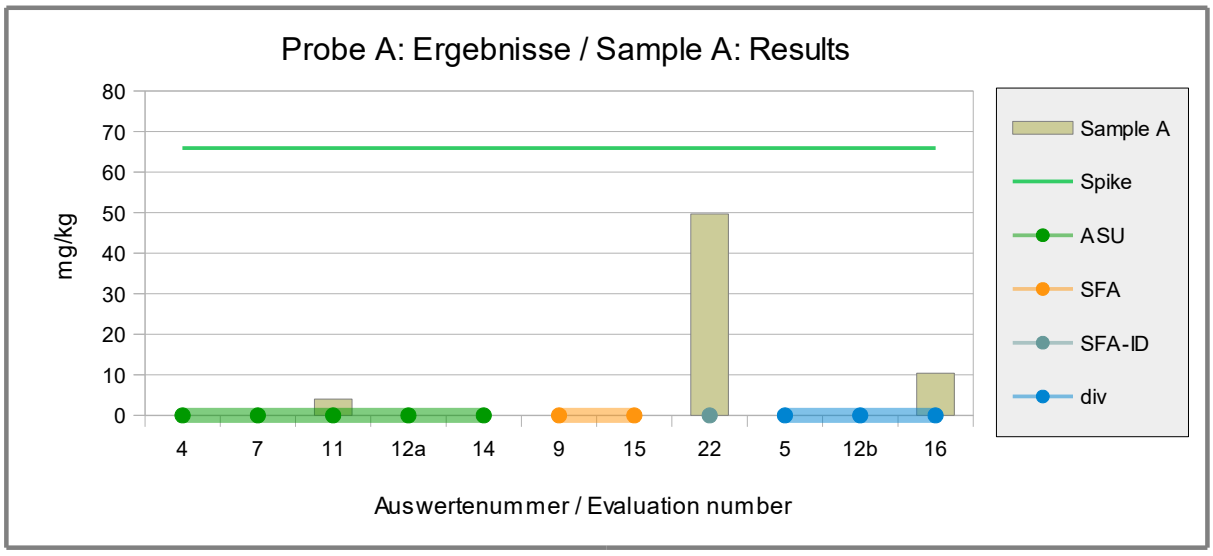


Abb./Fig. 9: PCR Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation of PCR: Spiking level sample

No quantitative valuation was done, because there were too few results available.

Evaluation number	Soya	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive			ASU	
7	positive			ASU	
11	positive	21,0		ASU	
12a	positive			ASU	
14	positive			ASU	
9	positive			SFA	
15	positive			SFA	
22	positive	43,6		SFA-ID	Given as soya DNA
5	positive			div	
12b	positive			div	
16	positive	84,7		div	

Number positive	11
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

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

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

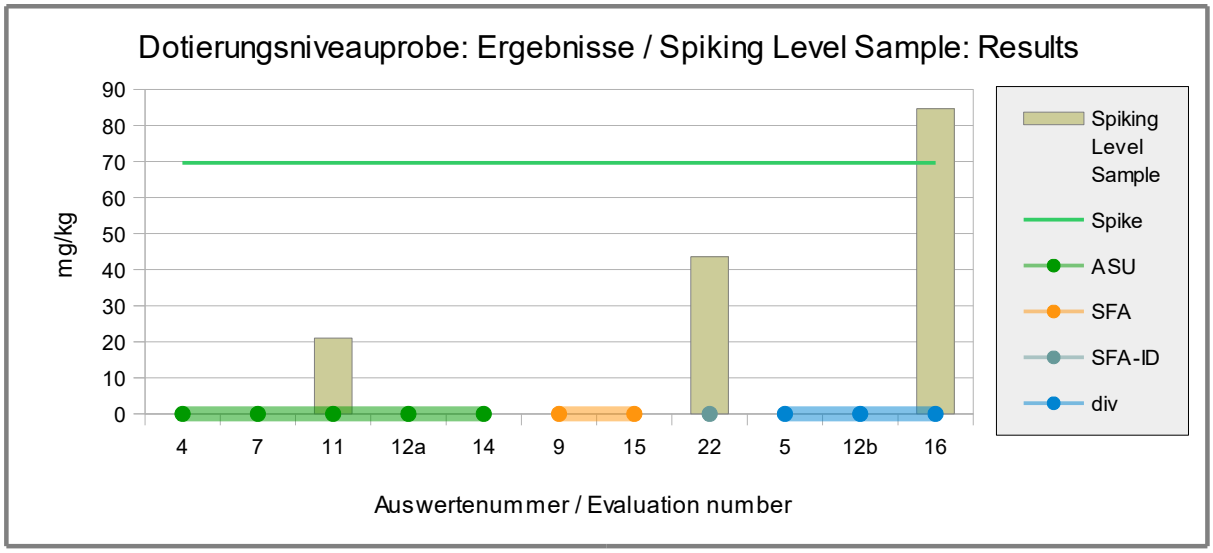


Abb./Fig. 10: PCR-Results soya
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
4							ASU	
7							ASU	
11	21,0	30	-2,8	4,00	6	-3,8	ASU	
12a							ASU	
14							ASU	
9							SFA	
15							SFA	
22	43,6	**63**	-1,5	49,6	**75**	-0,99	SFA-ID	Given as soya DNA (?)
5							div	
12b							div	
16	84,7	**122**	0,86	10,4	16	-3,4	div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**2**	Number in RA	**1**
Percent in RA	**67**	Percent in RA	**33**

* Recovery rate 100% relative size: soya/ soy flour, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Two of three participants obtained for the spiking level sample a recovery rate by PCR methods in the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A one recovery rate was in the range of acceptance.

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

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		
6	positive	27,0	negative	< LOQ	2/2 (100%)	AQ-G12	
8a	positive	26,0	negative	<LOD	2/2 (100%)	AQ-G12	
1	negative	<5	negative	<5	1/2 (50%)	AS-G12	
2	positive	45,0	negative	< 4	2/2 (100%)	IL	
20	positive	26,1	negative	<4,00	2/2 (100%)	IL	
23	positive	26,0	negative	< 1	2/2 (100%)	IL	Result converted °
3	positive	26,6	negative	<5,00	2/2 (100%)	RS	
4	positive	23,0	negative	< BG	2/2 (100%)	RS	
5a	positive	19,0	negative	<5	2/2 (100%)	RS	
7	positive	14,4	negative	<5,0	2/2 (100%)	RS	
8b	positive	20,0	negative	<LOD	2/2 (100%)	RS	
10a	positive	15,0	negative	<5	2/2 (100%)	RS	
12	positive	19,5	negative		2/2 (100%)	RS	
14	positive	22,1	negative		2/2 (100%)	RS	
16	positive	18,1	negative	<3,0	2/2 (100%)	RS	
17	positive	23,0	negative	<5	2/2 (100%)	RS	
19	positive	17,9	negative	<5	2/2 (100%)	RS	
21	positive	22,8	negative	<5	2/2 (100%)	RS	
22	positive	18,2	negative	<5	2/2 (100%)	RS	
24	positive	14,5	negative	<10	2/2 (100%)	RS-C	
11	positive	8,00	negative		2/2 (100%)	RS-F	
18	negative	10,4	negative	<10	1/2 (50%)	RS-F	Result Sample A at LOQ
10b	positive	17,0	negative	<2,5	2/2 (100%)	RS-S	
5b	positive	17,0	negative	<3,12	2/2 (100%)	SP-R5	
13	positive	14,7	negative	0	2/2 (100%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	23		0	
Number negative	2		25	
Percent positive	92		0	
Percent negative	8		100	
Consensus value	positive		negative	

Methods:

AQ-G12 = AgraQuant, RomerLabs
AS-G12 = AgraStrip (Lateral Flow), RomerLabs
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-S= Ridascreen® Fast sensitive, R-Biopharm
SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins
VT = Veratox, Neogen

Comment:

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

Note: Participant 18 classified the 10,4 mg/kg result for sample A as "negative", possibly because the value is below the amount to be labeled of 20 mg/kg.

Quantitative valuation of ELISA results: Sample A

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
6	27,0	1,4		AQ-G12	
8a	26,0	1,2		AQ-G12	
1	<5			AS-G12	
2	45,0	5,0		IL	
20	26,1	1,2		IL	
23	26,0	1,2		IL	Result converted °
3	26,6	1,3	1,4	RS	
4	23,0	0,61	0,63	RS	
5a	19,0	-0,19	-0,18	RS	
7	14,4	-1,1	-1,1	RS	
8b	20,0	0,01	0,02	RS	
10a	15,0	-0,99	-0,98	RS	
12	19,5	-0,09	-0,08	RS	
14	22,1	0,43	0,45	RS	
16	18,1	-0,37	-0,36	RS	
17	23,0	0,62	0,63	RS	
19	17,9	-0,41	-0,40	RS	
21	22,8	0,57	0,59	RS	
22	18,2	-0,35	-0,34	RS	
24	14,5	-1,1		RS-C	
11	8,00	-2,4		RS-F	
18	10,4	-1,9		RS-F	Result sample A at LOQ
10b	17,0	-0,59		RS-S	
5b	17,0	-0,59		SP-R5	
13	14,7	-1,1		VT	

° calculation see p. 19

Methods:

AQ-G12 = AgraQuant, RomerLabs
AS-G12 = AgraStrip (Lateral Flow), RomerLabs
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-S= Ridascreen® Fast sensitive, R-Biopharm
SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins
VT = Veratox, Neogen

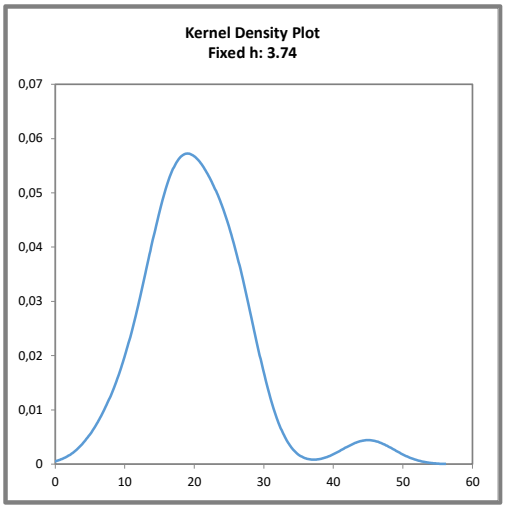


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetric distribution of results with a secondary peak at approx. 45 mg/kg, due to a result out of the target range.

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	24	13
Number of outliers	-	0
Mean	20,5	20,0
Median	19,3	19,5
Robust Mean (X_{pt})	**20,0**	**19,9**
Robust standard deviation (S*)	**5,83**	**3,72**
Target range:		
Target standard deviation σ_{pt}	**4,99**	**4,97**
lower limit of target range	**10,0**	**9,94**
upper limit of target range	**29,9**	**29,8**
Quotient S/σ_{pt}*	*1,2*	*0,75*
Standard uncertainty $U_{(Xpt)}$	*1,49*	*1,29*
Results in the target range	*22*	*13*
Percent in the target range	*92*	*100*

Method:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation shows nearly a symmetric distribution (a high single value).

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

The robust means of the evaluations were 110% each of the spiking level of gluten to sample A and in the range of the recommendations for the applied methods (s. 3.4.3 and p.46 "Recovery rates ELISA for gluten").

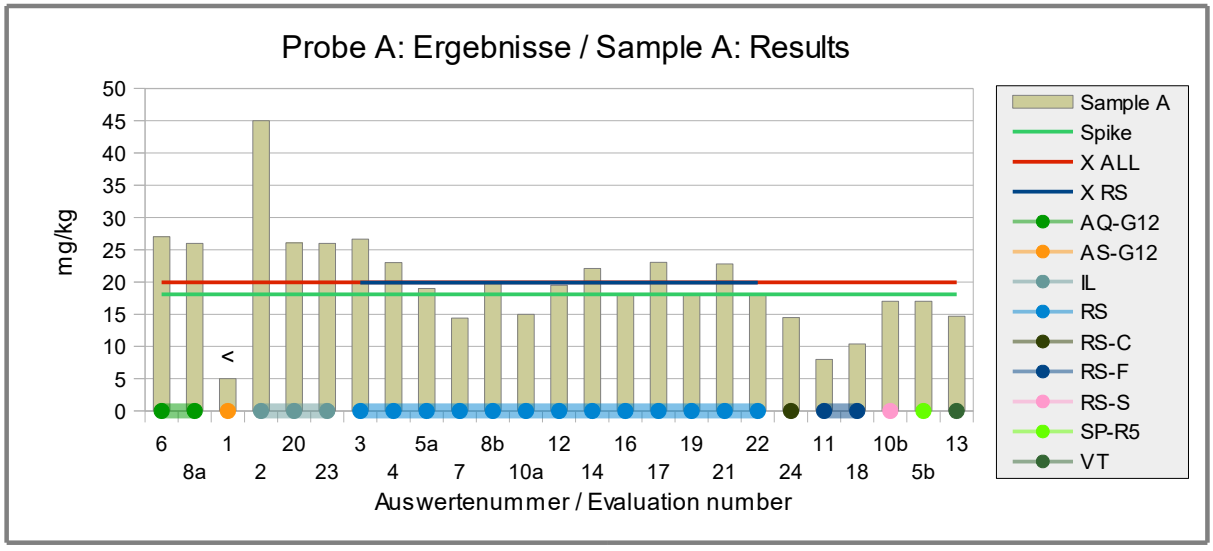


Abb./Fig. 12: ELISA Results gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

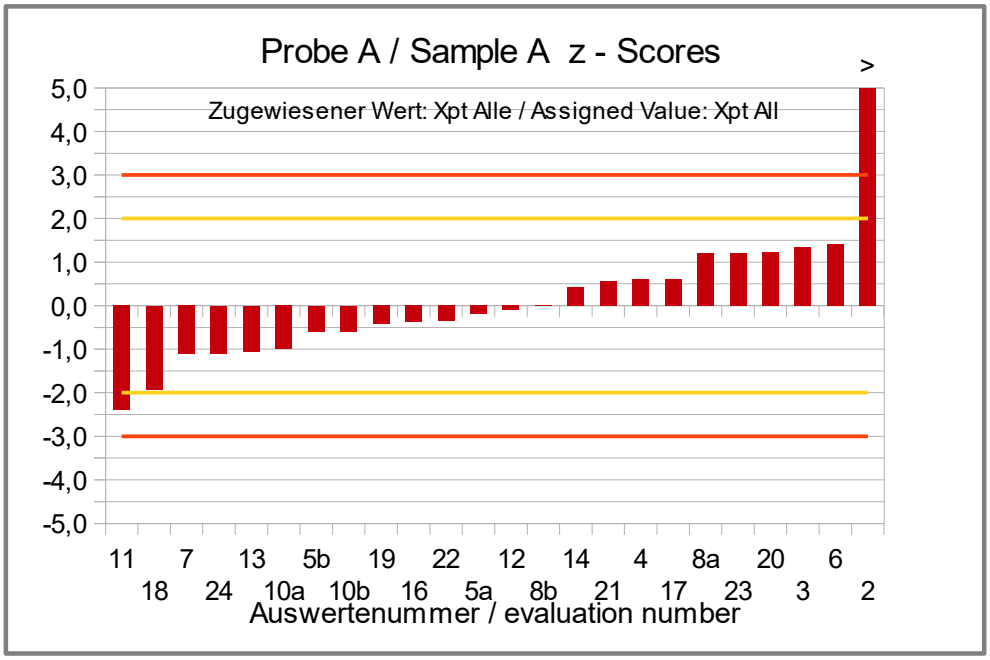


Abb./Fig. 13:
z-Scores ELISA Results gluten
Assigned value median of all results

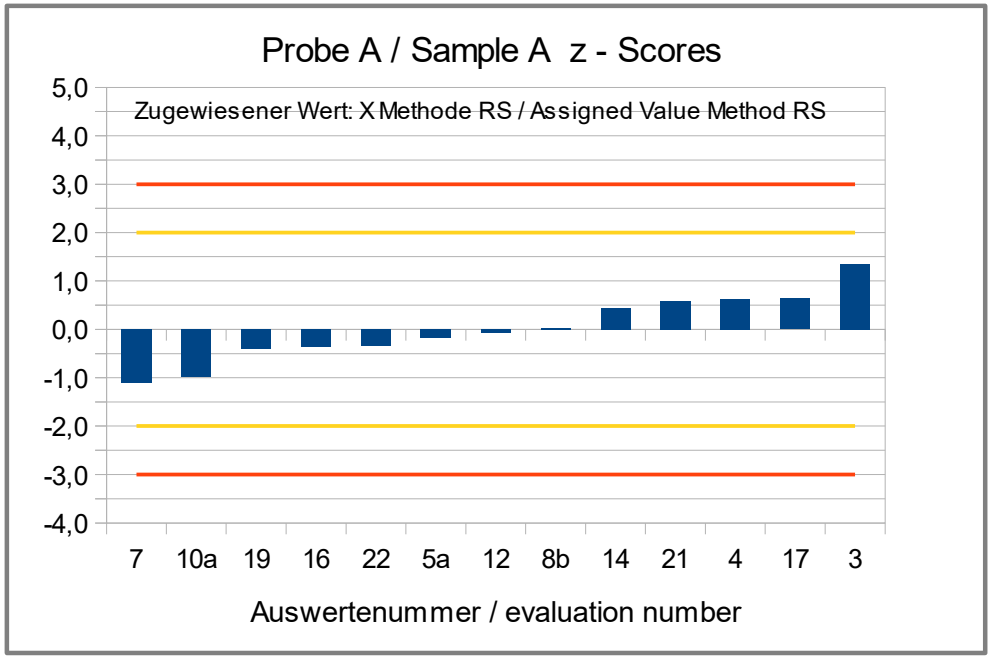


Abb./Fig. 14:
z-Scores ELISA Results gluten Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
6	49,0	0,37		AQ-G12	
8a	45,0	0,01		AQ-G12	
1	<5			AS-G12	
2	94,4			IL	Result excluded
20	176			IL	Result excluded
23	148			IL	Result converted Result excluded
3	56,2	1,0	0,88	RS	
4	43,6	-0,12	-0,21	RS	
5a	53,0	0,72	0,60	RS	
7	46,4	0,13	0,03	RS	
8b	51,0	0,54	0,43	RS	
10a	26,0	-1,7	-1,7	RS	
12	39,1	-0,52	-0,60	RS	
14	43,5	-0,12	-0,22	RS	
16	48,2	0,29	0,19	RS	
17	40,5	-0,39	-0,48	RS	
19	39,0	-0,53	-0,61	RS	
21	57,2	1,1	0,96	RS	
22	47,0	0,18	0,08	RS	
24	58,5	1,2		RS-C	
11	25,0	-1,8		RS-F	
18	38,3	-0,59		RS-F	
10b	>20			RS-S	
5b	42,0	-0,26		SP-R5	
13	37,5	-0,66		VT	

° calculation see p. 19

Methods:

AQ-G12 = AgraQuant, RomerLabs
AS-G12 = AgraStrip (Lateral Flow), RomerLabs
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-S= Ridascreen® Fast sensitive, R-Biopharm
SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins
VT = Veratox, Neogen

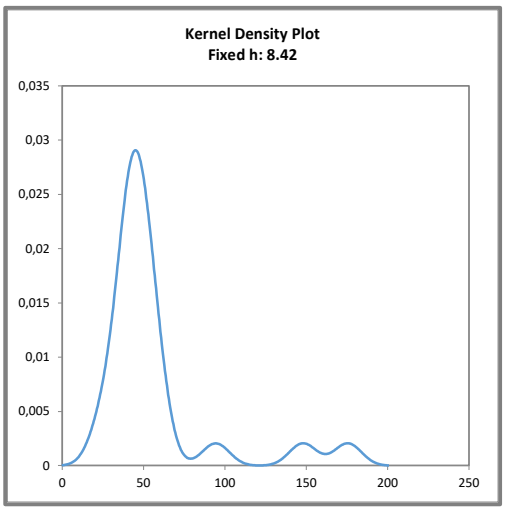


Abb. / Fig. 15:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetric distribution of results with several secondary peaks at 90-180 mg/kg, due to results of the method IL.

Characteristics: Quantitative evaluation ELISA gluten

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	20°	13
Number of outliers	3	0
Mean	44,3	45,4
Median	44,3	46,4
Robust Mean (X_{pt})	**44,9**	**46,1**
Robust standard deviation (S*)	**8,74**	**7,93**
Target range:		
Target standard deviation σ_{pt}	**11,2**	**11,5**
lower limit of target range	**22,4**	**23,0**
upper limit of target range	**67,3**	**69,1**
Quotient S/σ_{pt}*	*0,78*	*0,69*
Standard uncertainty $U_{(Xpt)}$	*2,44*	*2,75*
Results in the target range	*20*	*13*
Percent in the target range	*100*	*100*

° *without results no. 2, 20 and 23 (method IL excluded in advance)*

Methoden:
RS = R-Biopharm, Ridascreen®

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a method-dependent difference of the method IL. Therefore the results were not considered for the quantitative evaluation.

The evaluation of the results of all methods as well as the results of method RS showed a low variability. The quotients S/σ_{pt} were below 1,0. The robust standard deviations were in the lower range of established values for the repeatability and reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given.*
This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 126% and 129% of the spiking level of gluten to the spiking level sample and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.46 "Recovery rates ELISA for gluten").

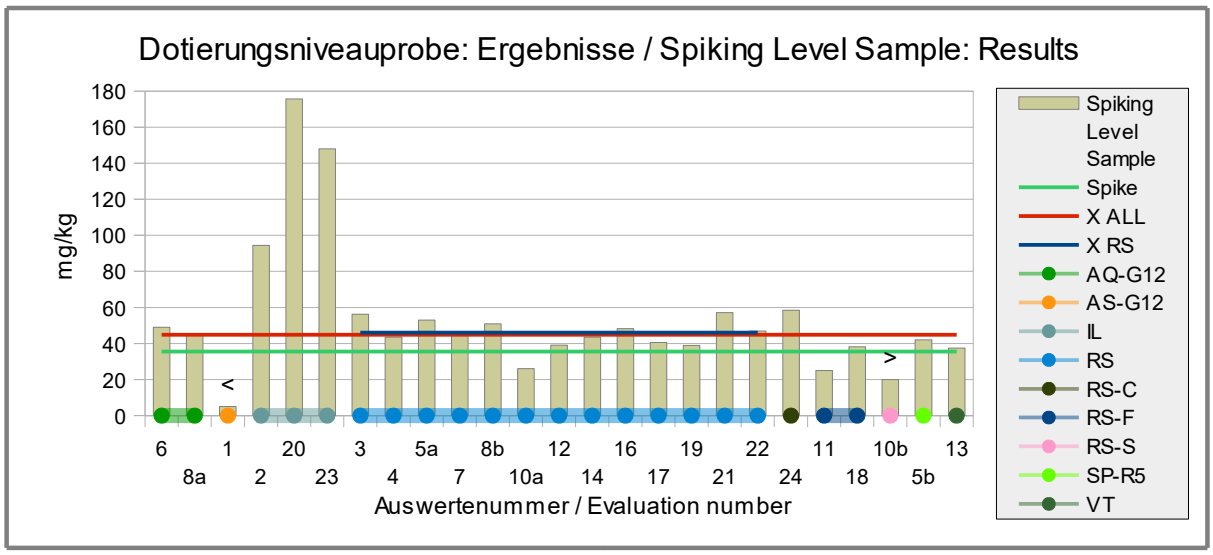


Abb./Fig. 16: ELISA Results gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS
round symbols = Applied methods (see legend)

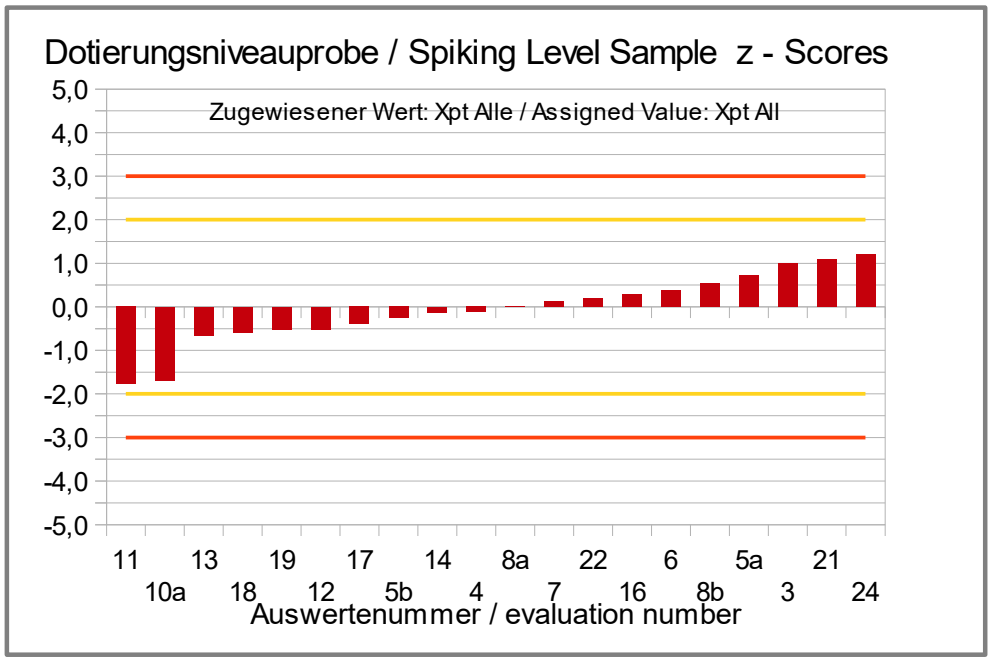


Abb./Fig. 17:
z-Scores ELISA Results gluten
Assigned value robust mean of all results

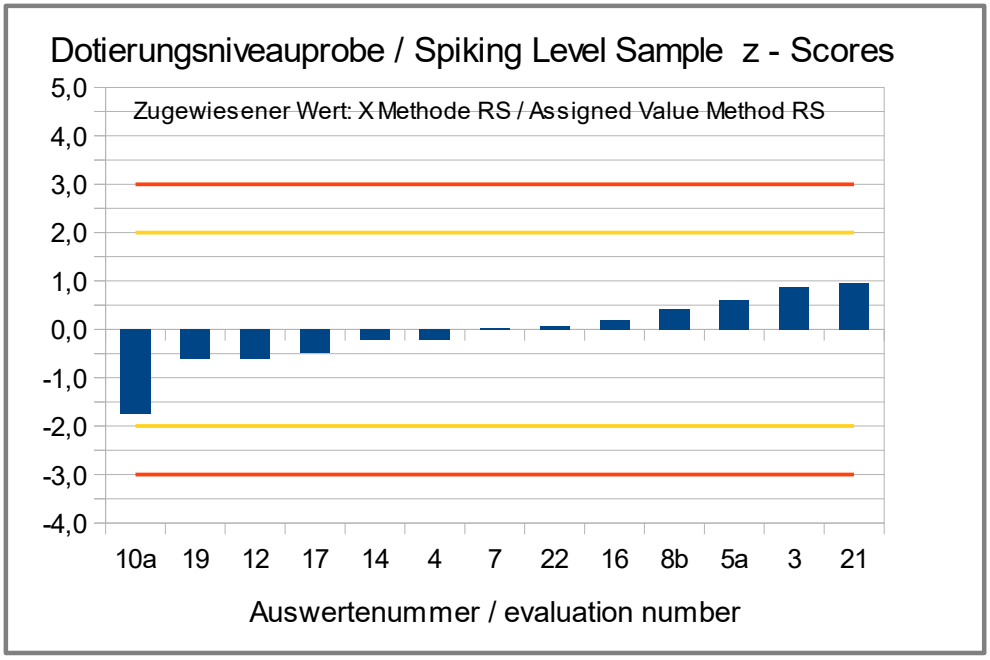


Abb./Fig. 18:
z-Scores ELISA Results gluten Assigned value robust mean of results method RS (R-Biopharm, Ridascreen)

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

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
6	49,0	**138**	1,5	27,0	**149**	2,0	AQ-G12	
8a	45,0	**126**	1,1	26,0	**144**	1,7	AQ-G12	
1	<5			<5			AS-G12	
2	94,4	265	6,6	45,0	249	5,9	IL	
20	176	493	16	26,1	**144**	1,8	IL	
23	148	416	13	26,0	**144**	1,7	IL	Result converted °
3	56,2	158	2,3	26,6	**147**	1,9	RS	
4	43,6	**122**	0,90	23,0	**127**	1,1	RS	
5a	53,0	**149**	2,0	19,0	**105**	0,20	RS	
7	46,4	**130**	1,2	14,4	**80**	-0,82	RS	
8b	51,0	**143**	1,7	20,0	**110**	0,42	RS	
10a	26,0	**73**	-1,1	15,0	**83**	-0,69	RS	
12	39,1	**110**	0,39	19,5	**108**	0,31	RS	
14	43,5	**122**	0,89	22,1	**122**	0,88	RS	
16	48,2	**135**	1,4	18,1	**100**	0,00	RS	
17	40,5	**114**	0,55	23,0	**127**	1,1	RS	
19	39,0	**110**	0,38	17,9	**99**	-0,04	RS	
21	57,2	161	2,4	22,8	**126**	1,0	RS	
22	47,0	**132**	1,3	18,2	**101**	0,02	RS	
24	58,5	164	2,6	14,5	**80**	-0,80	RS-C	
11	25,0	**70**	-1,2	8,00	44	-2,2	RS-F	
18	38,3	**108**	0,30	10,4	**57**	-1,7	RS-F	Result at the LOQ
10b	>20			17,0	**94**	-0,24	RS-S	
5b	42,0	**118**	0,72	17,0	**94**	-0,24	SP-R5	
13	37,5	**105**	0,21	14,7	**81**	-0,75	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**17**	Number in RA	**22**
Percent in RA	**74**	Percent in RA	**92**

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ-G12 = AgraQuant, RomerLabs
AS-G12 = AgraStrip (Lateral Flow), RomerLabs
IL = Immunolab
RS = Ridascreen®, R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
RS-S= Ridascreen® Fast sensitive, R-Biopharm
SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins
VT = Veratox, Neogen

Comments:

74% (17) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample A 92% (22) of the obtained recovery rates were within the recommended range.
The related z-scores are based on the target standard deviation of 25%.

4.2.2 PCR Results: wheat (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		
12	positive		negative		2/2 (100%)	ASU	
15	positive		positive		1/2 (50%)	SFA	Sample A positive in traces
22a	positive	15,1	negative	<1	2/2 (100%)	SFA-ID	Given as 'gluten containing cereal'
22b	positive	14,0	negative	<1	2/2 (100%)	SFA-ID	Given as 'wheat'
5	positive		negative		2/2 (100%)	div	
11	positive	3,00	negative		2/2 (100%)	div	
14	positive		negative		2/2 (100%)	div	Sample A positive in traces

	Sample A		Sample B	
Number positive	7		1	
Number negative	0		6	
Percent positive	100		14	
Percent negative	0		86	
Consensus value	positive		negative	

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

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

Quantitative valuation of PCR: Sample A

No quantitative valuation was done, because there were too few results available.

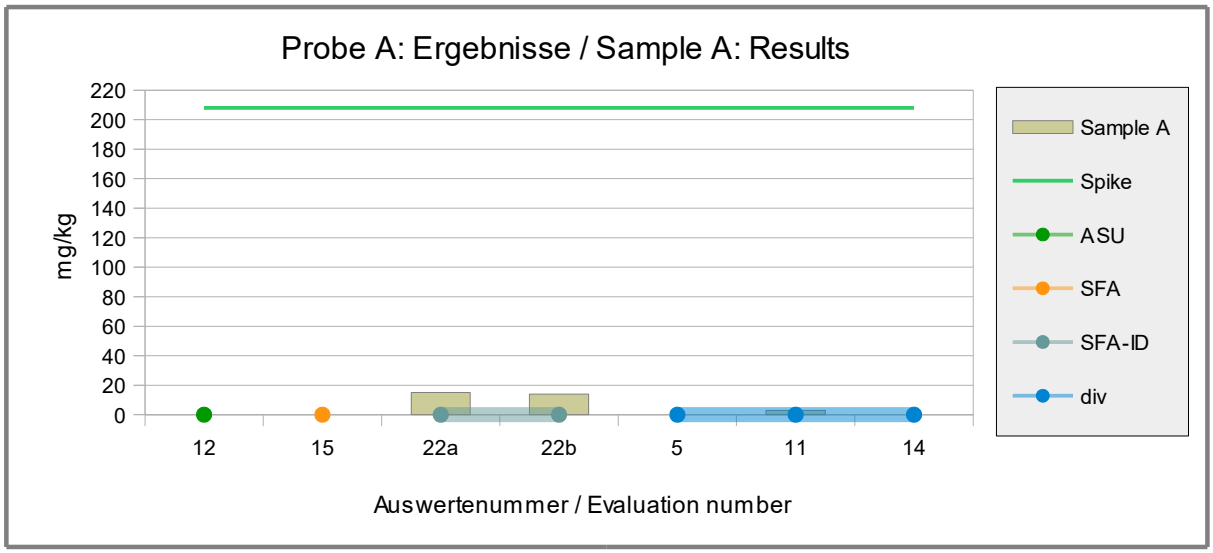


Abb./Fig. 19: PCR Results gluten-containing cereal - wheat
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation of PCR: Spiking level sample

No quantitative valuation was done, because there were too few results available.

Evaluation number	Gluten-containing Cereals	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
12	positive			ASU	
15	positive			SFA	
22a	positive	107		SFA-ID	Given as 'gluten-containing cereal'
22b	positive	140		SFA-ID	Given as 'wheat'
5	positive			div	
11	positive	780		div	
14	positive			div	

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

Methods:

ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

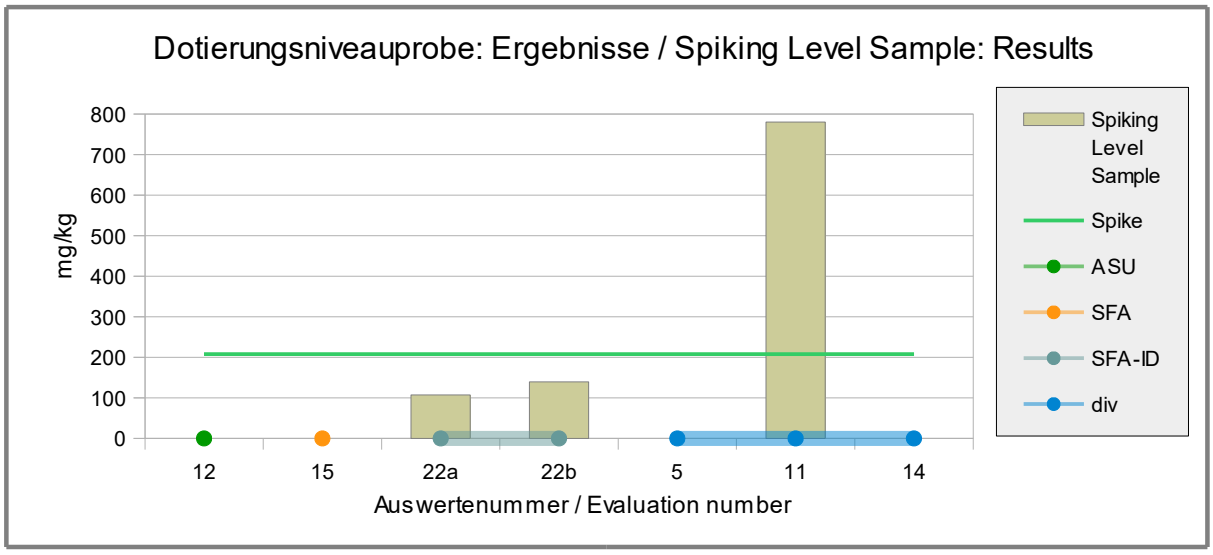


Abb./Fig. 20: PCR-Results gluten-containing cereals - wheat
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores PCR for wheat (gluten): Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
12							ASU	
15							SFA	
22a	107	26	-3,0	15,1	7,3	-3,7	SFA-ID	Given as 'gluten-containing cereal'
22b	140	34	-2,6	14,0	6,7	-3,7	SFA-ID	Given as 'wheat'
5							div	
11	780	191	3,6	3,00	1,4	-3,9	div	
14							div	

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

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

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

None of the participants obtained for the spiking level sample or the processed spiked food matrix sample A a recovery rate by PCR methods in the range of the AOAC-recommendation of 50-150%.

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

4.2.3 PCR Results: other

Qualitative valuation of results

Evaluation number	Sample A	Sample B	Spiking Level Sample	Method	Remarks
	pos/neg	pos/neg	[mg/kg]		
12	positive	positive	negative	div	Buckw heat
12	negative	negative	negative	div	Barley
12	negative	negative	negative	div	Oats
12	negative	negative	negative	div	Rye

Methods:

div = keine genaue Angabe / andere Methode

div = not indicated / other method

4.3 Participant z-Scores: overview table

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

Evalutation numver	ELISA Soya: **Xpt** (div. methods)		ELISA Soya: **Xpt** (method: RS-F)		ELISA Gluten: **Xpt** (div. methods)		ELISA Gluten: **Xpt** (method: RS)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	-	-	-	-	-	-	-	-
2	-	-2,6	-	-	5,0	-	-	-
3	-	-	-	-	1,3	1,0	1,4	0,88
4	0,41	-0,64	0,69	-0,32	0,61	-0,12	0,63	-0,21
5 / 5a	0,15	-2,0	-	-	-0,19	0,72	-0,18	0,60
5b	-	-	-	-	-0,59	-0,26	-	-
6	-	2,3	-	-	1,4	0,37	-	-
7	-0,43	-0,51	-0,21	-0,19	-1,1	0,13	-1,1	0,03
8 / 8a	-	3,1	-	-	1,2	0,01	-	-
8b	-	-	-	-	0,01	0,54	0,02	0,43
9 / 9a	-1,3	-	-1,1	-	-	-	-	-
9b	-	-	-	-	-	-	-	-
10 / 10a	-2,9	-	-2,8	-3,4	-0,99	-1,7	-0,98	-1,7
10b	-	-	-	-	-0,59	-	-	-
11	-	-	-	-	-2,4	-1,8	-	-
12	1,3	0,17	1,6	0,56	-0,09	-0,52	-0,08	-0,60
13	-	2,9	-	-	-1,1	-0,66	-	-
14	0,25	0,10	0,52	0,48	0,43	-0,12	0,45	-0,22
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-0,37	0,29	-0,36	0,19
17	-	-	-	-	0,62	-0,39	0,63	-0,48
18	-	-	-	-	-1,9	-0,59	-	-
19	0,77	-0,10	1,1	0,26	-0,41	-0,53	-0,40	-0,61
20	-	-	-	-	1,2	-	-	-
21	-0,53	-0,67	-0,31	-0,36	0,57	1,1	0,59	0,96
22 / 22a	-0,60	0,06	-0,38	0,44	-0,35	0,18	-0,34	0,08
22b	-	-1,1	-	-	-	-	-	-
23	2,5	-0,32	-	-	1,2	-	-	-
24	-	-	-	-	-1,1	1,2	-	-

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

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

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

Evaluation number	ELISA Soya: Xpt (div. methods)		ELISA Gluten: Xpt (div. methods)		PCR Soya: Xpt (div. methods)		PCR Gluten: Xpt (div. methods)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	-	-	-	-	-	-	-	-
2	-3,9	-1,7	5,9	6,6	-	-	-	-
3	-	-	1,9	2,3	-	-	-	-
4	-0,95	1,4	1,1	0,90	-	-	-	-
5 / 5a	-1,1	-0,77	0,20	2,0	-	-	-	-
5b	-	-	-0,24	0,72	-	-	-	-
6	-	6,2	2,0	1,5	-	-	-	-
7	-1,5	1,7	-0,82	1,2	-	-	-	-
8 / 8a	-3,5	7,6	1,7	1,1	-	-	-	-
8b	-	-	0,42	1,7	-	-	-	-
9 / 9a	-2,1	-	-	-	-	-	-	-
9b	-	-	-	-	-	-	-	-
10 / 10a	-3,2	-3,1	-0,69	-1,1	-	-	-	-
10b	-	-	-0,24	-	-	-	-	-
11	-	-	-2,2	-1,2	-3,8	-2,8	-3,9	3,6
12	-0,4	2,8	0,31	0,39	-	-	-	-
13	-3,5	7,2	-0,75	0,21	-	-	-	-
14	-1,1	2,6	0,88	0,89	-	-	-	-
15	-	-	-	-	-	-	-	-
16	-	-	0,00	1,4	-3,4	0,86	-	-
17	-4,0	17	1,1	0,55	-	-	-	-
18	-	-	-1,7	0,30	-	-	-	-
19	-0,70	2,3	-0,04	0,38	-	-	-	-
20	-4,0	-3,7	1,8	16	-	-	-	-
21	-1,6	1,4	1,0	2,4	-	-	-	-
22 / 22a	-1,6	2,6	0,02	1,3	-0,99	-1,5	-3,7	-3,0
22b	-4,0	0,7	-	-	-	-	-3,7	-2,6
23	0,48	2,0	1,7	13	-	-	-	-
24	-3,5	-	-0,80	2,6	-	-	-	-

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

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Soya

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	ELISA Test-Kit+Manufacturer
AQ	6	04.03.20	negative	< LOQ	negative	< LOQ	positive	60	0,87	2,2	40	soy protein	AgraQuant ELISA Soy COKAL0448, RomerLabs
AQ	8	27.03.20	positive	2,6	negative	<LOD	positive	68	0,87	2,18	40	Soyprotein	AgraQuant ELISA Soy COKAL0448, RomerLabs
AT	17	23.04.20	negative	0,02	negative	-4,13	positive	124,48	9,5	114		Soyprotein	AlerTox Soy (STI) ELISA, Biomedal
IL-SP	23	25.02.20	positive	25	negative	< 0.5	positive	35	0.2	2		Soyprotein	SENSISpec Soy Protein Total ELISA
IL-STI	2	26.02.20	positive	0,024	negative	< 0,04	positive	0,94	0,04			Soyprotein	Immunolab Soy ELISA
IL-STI	20	19.03.20	positive	0,05	negative	<0,04	positive	1,54	0,016	0,04		Soyprotein	Immunolab Soy ELISA
MI-II	5	28.02.	positive	16	negative	<0,31	positive	19	0,31	0,31		Soyprotein	Morinaga Soya ELISA Kit II
RS-F	4	03.03.20	-	17	-	< BG	-	32		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	7	27.02.20	positive	13,76	negative	<2,5	positive	33,2	0,24	2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	9a		positive	10,4	negative	< 2,5	positive	> 20		2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	10	20.03.20	positive	13	negative	<2,5	positive	16		2,5		Soyflour	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	12	3.+17.03.2020	positive	20,3	negative		positive	39,7	0,31	2,5	25	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	14	13.03.	positive	16,4	negative		positive	39	2,5	2,5	50	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	19	29.04.	-	18,4	-	5,7	positive	37,1	0,24	2,5	63,1	Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	21	15.04.	positive	13,38	negative	<2,5	positive	31,71	0,24	2,5		Soyprotein	Ridascreen® FAST Soya R7102, R-Biopharm
RS-F	22a	05.03.20	positive	13,12	negative	<2.5	positive	38,64	2,5	2,5		Soy Protein	Ridascreen® Fast Soya R7102, R-Biopharm
VT	9b		-		negative	< 1,17	positive	> 11,8		1,17		Soyprotein	Veratox Soy Allergen, Neogen
VT	13	19.03.20	-	2,8	-	0	-	66				Protein	NEOGEN Veratox Soja Allergen Test
VT	22b	05.03.2020	negative	<2.5	negative	<2.5	positive	81	2,5	2,5		Soyflour	Veratox Soy Allergen, Neogen
VT	24	16.03.20	-	2,53	-	<2.5	-	>25		2,5		Protein	Selection Soya-Kits: Neogen Veratox

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

Continuation ELISA Soya:

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	6	STI	aqueous buffer/15 minutes/ 60°C	no	
AQ	8			yes	
AT	17				
IL-SP	23				Conversion factor for roasted soy flour: 2.2 --> 55 ppm for sample A
IL-STI	2				In order to obtain the content of an underlying raw product from the determined STI content, the result must be multiplied by a corresponding conversion factor (F). (Unroasted soy flour: 42, roasted soy flour: 470)
IL-STI	20	STI	extractionbuffer(kit provided) /15min/ 60 C	YES	
MI-II	5	recognizes the soy protein beta-conglycinin	according to manufacturer's instructions	yes	M2117
RS-F	4	Antibodies specifically recognize heated soy proteins	according to test instructions	yes	
RS-F	7		according to test kit description	yes	
RS-F	9a			yes	
RS-F	10				
RS-F	12	The antibodies used recognize specifically heated soy proteins.	according to kit	yes	--
RS-F	14	Soyaprotein		yes	
RS-F	19			yes	
RS-F	21	AB for heated soy proteins	according to test kit instructions	no	
RS-F	22a	As Per Kit Instructions	As Per Kit Instructions	No	
VT	9b			yes	
VT	13	Soya	15 min / 60°C		
VT	22b	As Per Kit Instructions	As Per Kit Instructions	Yes	
VT	24			Yes	

5.1.2 ELISA: Gluten

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	ELISA Test-Kit+Manufacturer
AQ-G12	6	04.03.20	positive	27	negative	< LOQ	positive	49	2	4	40	Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
AQ-G12	8a	26.03.20	positive	26	negative	<LOD	positive	45	2	4	40	Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
AS-G12	1		negative	5–20	negative	5–20	positive	5–20	5ppm	5–20ppm		Gluten	Glutenschnelltest Agrastrip Allergen Gluten G12 (Romer Labs)
IL	2	26.02.20	positive	45	negative	< 4	positive	94,4	4			Gluten	Immunolab Gliadin/Gluten ELISA
IL	20	19.03.20	positive	26,06	negative	<4,00	positive	175,56	0,6	4		Gluten	Immunolab Gliadin/Gluten ELISA
IL	20	04.05.20	positive	78,66	negative		positive	186,17				Gluten	Immunolab Gliadin/Gluten ELISA
IL	23	14,04.20	positive	13	negative	< 0.5	positive	74	0.3	2		Gliadin	Immunolab Gliadin/Gluten ELISA
RS	3	18.03.20	positive	26,63	negative	<5,00	positive	56,17	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	4	26/27/02/20	-	23	-	< BG	-	43,6		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	5a	25.02.	positive	19	negative	<5	positive	53	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	7	03.03.20	positive	14,4	negative	<5,0	positive	46,35	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8b	03.11.20	positive	20	negative	<LOD	positive	51	1	5	50	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	10a		positive	15	negative	<5	positive	26		5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	12	2.+12.3.20	positive	19,5	negative		positive	39,1	1	5	25	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	14	19.03.	positive	22,1	negative		positive	43,5	5	5	50	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	16	03.03.20	-	18,1	-	<3.0	-	48,2				Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	17	24.04.20	positive	23,04	negative	<5	positive	40,52	5	80		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	19	26.03.	-	17,9	-	<5	positive	39	1	5	52	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	21	15.04.	positive	22,8	negative	<5	positive	57,15	1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	22	23.03.2020	positive	18,21	negative	<5	positive	46,95	5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS-C	24	15.03.20	-	14,5	-	<10	-	58,5		10		Protein	Selection Gluten-Kits: r-biopharm Ridascreen competitive
RS-F	11		positive	8	negative		positive	25	5	10	50	Please select!	Ridascreen® FAST Gliadin R7002, R-Biopharm
RS-F	18	29.04.20	negative	10,38	negative	<10	positive	38,28	1	10		Gluten	Ridascreen® FAST Gliadin R7002, R-Biopharm
RS-S	10b		positive	17	negative	<2,5	positive	>20		2,5		Gluten	Ridascreen® Fast Gliadin Sensitive R7051, R-Biopharm
SP-R5	5b	28.02.	positive	17	negative	<3,12	positive	42	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins
VT	13	19.03.20	-	14,7	-	0	-	37,5				Protein	NEOGEN Veratox Gliadin R5

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Gluten:

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ-G12	6	Gluten	Extraction buffer/ 40 minuts 50°C/ethanol /60 minuts orbital shaker	no	
AQ-G12	8a			yes	
AS-G12	1				
IL	2				
IL	20		40% ethanol / 5min/ room temperature	YES	
IL	20		Immunolab: same as above (email from 6/5/2020)	no	
IL	23				
RS	3	R5		yes	Accreditation according to ISO 17025 has taken place, the decision is still pending
RS	4	R 5	accordin to kit instruction	yes	
RS	5a	R5 Mendez, detects prolamines from wheat, rye and barley	according to manufacturer's instructions	yes	
RS	7		according to test kit description	yes	
RS	8b			yes	
RS	10a				
RS	12	R5	according to kit	yes	--
RS	14	Gliadine (R5-Antibody)		yes	
RS	16				
RS	17				
RS	19		Preparation with cocktail R7006	yes	
RS	21	R5	according to test kit instructions	no	
RS	22	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-C	24			Yes	
RS-F	11		according to manual	yes	
RS-F	18	Peroxidase-coupled R5 antibody	Rida Extraction Solution (colorless) Art. No. R7098 /method according to R-biopharm's instructions	no	
RS-S	10b				
SP-R5	5b	R5 Mendez, detects prolamines from wheat, rye and barley	according to manufacturer's instructions	yes	
VT	13	Gluten	40 min / 50°C		

5.1.3 PCR: Soya

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	PCR Test-Kit+Manufacturer
ASU	4	27.02.20	positive		negative		positive					Soya-DNA	ASU §64 Methode/method
ASU	7	12.03.20	positive		negative		positive					Soya-DNA	ASU §64 Methode/method
ASU	11		positive	4	negative		positive	21	5	10	30	Please select!	Selection PCR methods
ASU	12a	03.03.20	positive		negative		positive					Soya-DNA	ASU §64 Methode/method
ASU	14	22.04.20	positive		negative		positive					Soya-DNA	ASU L 00.00-105
SFA	9		positive		negative		positive		0,4			Soya-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	25.02.20	positive		positive		positive		0,4			Soya-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	22	17.04.20	positive	49,64	negative	<1	positive	43,56	1	1		Soya-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	5	27.02.	positive		negative		positive		10			Soya DNA_	internal method
div	12b	03.03.20	positive		negative		positive					Soya-DNA	QT-EVE-GM-009, 2013-01
div	16	19.04.20	-	10,4	-	<2.5	-	84,65				Soyflour	house method

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4	Soyes-Lectin-Gen 81bp	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
ASU	7	Lektin Gen Ie1 (74 bp)	Extraction according to ASU §64 LFGB L15.05-1 (SDS/guanidinium chloride buffer with proteinase K, purification using wizard kit from Promega); Real-time PCR with 45 cycles	yes	
ASU	11	lectin	Wizard/Realtime PCR	yes	
ASU	12a	Lectin-Gen 81 Bp	Maxwell RSC Pure Food GMO and Authentication KIT	yes	4-plex
ASU	14		Wizard-DNA-Präparation / Realtime PCR, 45 cycles		
SFA	9			yes	
SFA	15				
SFA-ID	22	As Per Kit Instructions	As Per Kit Instructions	No	
div	5		CTAB, Proteinase K / Promaga Wizard DNA CleanUp / Real-time PCR 45 cycles	yes	
div	12b	Lectin-Gen 74 Bp	Maxwell RSC Pure Food GMO and Authentication KIT	yes	1-plex PCR
div	16				

5.1.4 PCR: Wheat (gluten)

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Level Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	PCR Test-Kit+Manufacturer
ASU	12	03.03.20	positive		negative		positive					Wheat-DNA	ASU §64 Methode/method
SFA	15	25.02.20	traces		positive		positive		0,4			gluten free cereals-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	22a	05.03.20	positive	15,08	negative	<1	positive	107,14	1	1		gluten free cereal	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	22b	05.03.20	positive	14	negative	<1	positive	139,51	1	1		wheat	Sure Food Allergen ID, R-Biopharm / Congen
div	5	27.02.	positive		negative		positive		40			Wheat DNA_	internal method
div	11		positive	3	negative		positive	780	5	10	30	Please select	Selection PCR methods
div	14	22.04.20	traces positive		negative		positive					Wheat-DNA	Alary et al. 2002

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	12	Glutenin system of wheat and rye	Maxwell RSC Pure Food GMO and Authentication KIT	yes	1-plex PCR
SFA	15				
SFA-ID	22a	As Per Kit Instructions	As Per Kit Instructions	No	
SFA-ID	22b	As Per Kit Instructions	As Per Kit Instructions	No	
div	5		CTAB, Proteinase K / Promaga Wizard DNA CleanUp / Real-time PCR 45 cycles	yes	
div	11	2020	Wizard/Realtime PCR	yes	
div	14		Wizard-DNA-Präparation / Realtime PCR, 45 cycles		

5.1.5 PCR: Other

Parameter	Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
PCR-Results			day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	PCR Test-Kit+Manufacturer
Buckwheat	div	12	04.03.20	positive		positive		negative					Buckwheat-DNA	Yamakawa et al.: Biosci. Biotechnol. Biochem. 72 (8), 2228-2231, 2008
Barley	div	12	09.03.20	negative		negative		negative					Barley-DNA	Dolch et al.; Food Control 101 (2019) 180-188
Oat	div	12	09.03.20	negative		negative		negative					Oat-DNA	Dolch et al.; Food Control 101 (2019) 180-188
Rye	div	12	09.03.20	negative		negative		negative					Rye-DNA	Dolch et al.; Food Control 101 (2019) 180-188

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

Parameter	Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
PCR-Results			Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
Buckwheat	div	12	major allergenic storage protein	Maxwell RSC Pure Food GMO and Authentication KIT	yes	conv. PCR
Barley	div	12	γ-Hordein-Gen	Maxwell RSC Pure Food GMO and Authentication KIT		3-plex
Oat	div	12	12s seed storage protein-Gen	Maxwell RSC Pure Food GMO and Authentication KIT		3-plex
Rye	div	12	O-methyltransferase-Gen	Maxwell RSC Pure Food GMO and Authentication KIT		3-plex

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptA02 2020 Sample A

Weight whole sample	1,74	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	72	28,6
2	5,05	68	26,9
3	5,06	68	26,9
4	5,07	75	29,6
5	5,03	61	24,3
6	4,98	72	28,9
7	5,02	63	25,1
8	5,00	75	30,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,3	Particles
Standard deviation	5,28	Particles
χ^2 (CHI-Quadrat)	2,82	
Probability	**90**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	27,5	mg/kg
Standard deviation	2,10	mg/kg
rel. Standard deviaton	7,63	%
Horwitz standard deviation	9,71	%
HorRat-value	**0,79**	
Recovery rate	103	%

Microtracer Homogeneity Test
DLA ptA02 2020 Spiking Level Sample

Weight whole sample	1,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	64	25,5
2	4,98	65	26,1
3	5,02	66	26,3
4	4,97	61	24,5
5	4,96	63	25,4
6	4,98	69	27,7
7	4,99	69	27,7
8	4,98	67	26,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,5	Particles
Standard deviation	2,79	Particles
χ^2 (CHI-Quadrat)	0,83	
Probability	**100**	%
Recovery rate	125	%

Normal distribution		
Number of samples	8	
Mean	26,3	mg/kg
Standard deviation	1,12	mg/kg
rel. Standard deviaton	4,26	%
Horwitz standard deviation	9,78	%
HorRat-value	**0,43**	
Recovery rate	125	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL02 - 2020***
PT name	***Allergens II: Soya and Wheat ("Gluten") in „gluten-free“ Pastry***
Sample matrix (processing)	***Samples A + B:*** *"gluten free" Cookies (baked 150°C) / Ingredients: sugar, rice flour, corn starch, corn flour, eggs, rice starch, sunflower oil, butterfat, low-fat cocoa powder 1.7%, invert sugar syrup, shea butter, apple fiber, salt, raising agent: potassium tartrate, sodium carbonate, ammonium carbonate, thickener: guar gum, xanthan gum, cocoa , Flavors, acidifier: citric acid, antioxidant: rosemary extract, other food additives and allergenic foods soyflour and wheat flour (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each + 1 Spiking Level Sample: 15 g*
Storage	*Samples A, B + Spiking Level Sample: room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Soya (Soyprotein, 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***
Last Deadline	***the latest April 03rd 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*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
		Germany
		SWITZERLAND
		CANADA
		ITALY
		Germany
		SPAIN
		SWITZERLAND
		Germany
		Germany
		USA
		Germany
		Germany
		Germany
		SWITZERLAND
		Germany
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN
		GREECE
		AUSTRIA
		AUSTRIA
		AUSTRIA
		SPAIN

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

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

7. Index of references

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

munoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 16.01-9 Untersuchung von Lebensmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016) [Foodstuffs, determination of soya (Glycine max) in cereal flour by real-time PCR]
33. ASU §64 LFGB L 08.00-59 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
34. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2017) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]
35. ASU §64 LFGB L 08.00-66 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Weizen (Triticum L.) und Roggen (Secale cereale) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of wheat (Triticum L.) and rye (Secale cereale) in boiled sausages by real-time PCR]
36. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Informationen für Verbraucher über das Nichtvorhandensein oder das reduzierte Vorhandensein von Gluten in Lebensmitteln / on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food
37. Bruins-Slot et al. (2015) Evaluating the performance of gluten ELISA test kits: The numbers do not tell the tale, Cereal Chem 92(5):513-521
38. Köhler & Andersen (2014) Analyse von Glutengehalten in Getreide und getreidehaltigen Produkten, Tabellenwerk zum Nährstoffgehalt von Lebensmitteln 3.1.5.1, Deutsche Forschungsanstalt für Lebensmittelchemie Leibniz Institut Jahresbericht 2014 [Analysis of gluten contents in cereals and cereal products, nutrient tables of foods]
39. Allergen Data Collection - Update (2002): Soybean (Glycine max), Besler M., Helm R.M., Ogawa T., Internet Symposium on Food Allergens 2(Suppl.3): 1-35 (2000) http://www.food-allergens.de