DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

02/2016

Allergens II:

Soya and Wheat

in "gluten-free" Pastry

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1. Introduction

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

2. Realisation

2.1 Test material

Two PT-samples for the detection of allergens in the range of mg/kg and one spiking material sample were provided for analysis. The spiking material sample contains the respective allergenic ingredients in the range of 1-10 % and was added to the spiked PT-sample. The results of the spiking material 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 consists of a common in commerce "gluten-free" bread baking mixture. The basic composition of both sample A and sample B was the same baked bread (see table 1). The spiking material, which contains the allergenic ingredients soy flour and wheat flour, was baked separately as an ingredient in an aliquot of the same baking mixture 60 min at approximately 190°C. After cooling to room temperature and crushing this spiked bread was added to sample B. After pre-crushing the breads were dried for 1,5 hours at 70°C, crushed again, sieved (mesh: 2,0 mm) and homogenized.
The composition of the spiking material sample and the amounts of allergens in sample B is given in table 2.

After homogenisation the samples were portioned to approximately 25 g.

Table 1: Composition of DLA-Samples

Ingredients	Sample A	Sample B
Brown Bread, gluten free (baked 190°C, 60 min) Ingredients: Baking mixture (corn starch, flax seed flour, buckwheat flour, pea bran, rice bran, apple fiber, sugar, gelling agent: guar gum, salt), water, sunflower oil, dry yeast, salt Nutrients per 100g (only baking mixture): Protein 2.6 g, carbohydrates 63 g, fat 6.1 g	100 g/100g	66 g/100g *
Brown Bread, gluten free (baked 190°C, 60 min) Ingredients: Baking mixture (corn starch, flax seed flour, buckwheat flour, pea bran, rice bran, apple fiber, sugar, gelling agent: guar gum, salt), water, sunflower oil, dry yeast, salt Nutrients per 100g (only baking mixture): Protein 2.6 g, carbohydrates 63 g, fat 6.1 g	-	32 g/100g *
Spiking material sample	-	2,1 g/100g *

* related to total weight after baking of sample

Table 2: Added amounts of allergenic ingredients

Ingredients	Spiking material sample	Amounts in Sample B
Potato flour Nutrients per 100g: Protein 0 g	93 %	1,9 %
Soya: - as Soy flour - thereof Soyproteins	20100 mg/kg (2,01 %) 8040 mg/kg	413 mg/kg 165 mg/kg
Hazelnut spread	1,18 %	0,024 %
Skimmed milk powder	1,96 %	0,040 %
Wheat: - as Wheat flour Type 1050 - thereof total protein* - *thereof gluten***	15300 mg/kg (1,53 %) 1840 mg/kg 1650 mg/kg	314 mg/kg 37,7 mg/kg 33,9 mg/kg

* according to labelling

** Definition of "gluten" from the Gluten Intolerance Labelling Regulation (EU/41/2009) corresponds to 85-91% of wheat protein according to data from the literature

2.1.1 Homogeneity

Homogeneity of the spiking material sample and spiked sample B was checked by ELISA-test for gluten (fig. 1). The resulting standard deviations between the samples of < 15% ensured sufficient homogeneity [14, 15, 18, 19]. 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 (s. 3.8 and 3.11) [3].

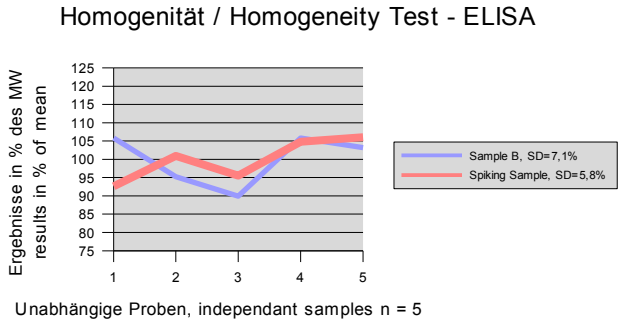

Fig. 1: Testing of homogeneity of DLA-sample B and spiking material sample. Results are given in percent of the arithmetic mean

2.2 Test

The portions of test material (sample A and sample B as well as the spiking material sample) were sent to every participating laboratory in the 5th week of 2016. The testing method was optional. The tests should be finished at March 18th 2016 the latest.

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. soyprotein and gluten in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specifity, 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.
One participant submitted no results and one participant delayed. All other participants submitted their results in time.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [21, 22, 23, 24]. It is for this reason that we contrast the results of the present proficiency test with several assigned values. Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.

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

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) („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].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. 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 identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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-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 (colloborative 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]:

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

Because in the present proficiency test the number of replicate measurements is n = 1, the reproducibility standard deviation σ_R is identical to the target standard deviation σ_{pt}.

The following table shows the relative reproducibility standard deviations from proficiency tests of ELISA-methods from German ASU §64 methods [25, 26, 27]:

Method	Parameter	Matrix	Mean values	Relative σ_R	Literature
ELISA	Soy protein	Sausage	0,36 - 4,07%	14 - 28%	L 06.00-56
ELISA (Manuf. A)	Peanut	Milk chocolate	5,9 - 174 mg/kg	20 - 31%	L 00.00-69
ELISA (Manuf. B)	Peanut	Milk chocolate	10,1 - 216 mg/kg	14 - 32%	L 00.00-69
ELISA (Manuf. A)	Peanut	Dark chocolate	5,7 - 148 mg/kg	22 - 33%	L 00.00-69
ELISA (Manuf. A)	Hazelnut	Dark chocolate	1,6 - 16,3 mg/kg	12 - 33%	L 44.00-7
ELISA (Manuf. A)	Hazelnut	Dark chocolate	2,4 - 21,3 mg/kg	14 - 19%	L 44.00-7

From these precision data of the ASU §64 methods the calculated relative target standard deviations are in the range of 12 - 33%.

For soya which is an analyte of the present proficiency test the relative reproducibility standard deviations were in the range of 14 - 28% in the matrix of sausage [25].

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 [20]. 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 [20].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA-test kits for the quantification of peanut [23}. 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%.

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 [18], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [15-17], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [19] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [14].

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 [14-20]	**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 [14]	**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 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. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and 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 Quotient S*/σ_{pt}

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

3.7 Standard uncertainty of the assigned value

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 Quotient $U_{(Xpt)}/\sigma_{pt}$ is reported in the characteristics of the test.

3.8 Figures

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

3.9 Recovery rates: Spiking

For the results of the spiking material 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 2. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of allergen-ELISAs proposed by the AOAC was used [19]. For quantitative PCR determinations we use the same range of acceptance.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.
The following result sections are structured equally for the allergenic components. First all results for a certain analyte are reported together for sample A and afterwards for sample B.

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

ELISA-results, which were given as soy flour or soybean, were converted into total soyprotein, when available with respect to the instructions of the test kit manufacturers. The original results are given in the documentation.
For soya a content of 40% protein in soy flour was assumed.

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

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.

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 X_{ALL}	z-Score $X_{M\,i}$	Method	Remarks
	pos/neg	[mg/kg]	X All	X Method i		

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

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

After that the recovery rates of the results for the spiking 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		
18	negative	< 1	positive	5	2/2 (100%)	IL	
2	negative		positive	841	2/2 (100%)	RS	outlier X_{all} and X_{RS}
4	negative	< 4	positive	52	2/2 (100%)	RS	
5	negative	< 2,5	positive	128,63	2/2 (100%)	RS	
6	negative		positive	124	2/2 (100%)	RS	
7	negative	< 2,5	positive	68,7	2/2 (100%)	RS	
10	negative	< 10	positive	51,76	2/2 (100%)	RS	
13a	negative	< 2.5	positive	76,36	2/2 (100%)	RS	
15	negative	< 2,5	positive	89,7	2/2 (100%)	RS	
19	negative	< 1,25	positive	> 20	2/2 (100%)	RS	
13b	negative	< 1,0	positive	< 1,0	2/2 (100%)	VT	Result converted *

* calculation see p. 14

	Sample A		Sample B	
Number positive	0		11	
Number negative	11		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT = Veratox, Neogen

Comments:

There were 100% negative results for sample A and 100% positive results for sample B by the ELISA-methods. One positive result was below the limit of quantification of the method (evaluation no. 13b, Veratox). The consensus values are in agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

Evaluation number	Soyprotein	z-Score Xpt_{ALL}	z-Score Xpt_{RS}	Method	Remarks
	[mg/kg]				
18	5	-3,8		IL	
2	841	35,6	31,7	RS	outlier X_{all} and X_{RS}
4	52	-1,6	-1,8	RS	
5	128,63	2,1	1,5	RS	
6	124	1,8	1,3	RS	
7	68,7	-0,8	-1,1	RS	
10	51,76	-1,6	-1,8	RS	
13a	76,36	-0,4	-0,8	RS	
15	89,7	0,2	-0,2	RS	
19	> 20			RS	
13b	< 1,0			VT	Result converted *

* calculation see p. 14

Methods:
IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT = Veratox, Neogen

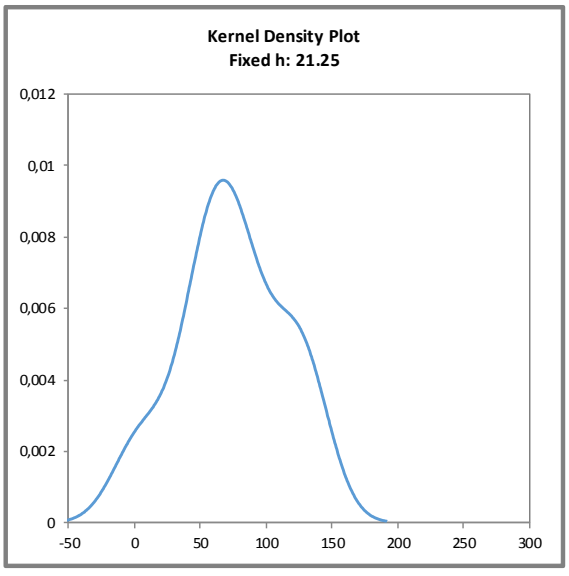

Fig. 2: Kernel Density Plot of all ELISA-results soyprotein without outliers (with h = σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a normal distribution with two shoulders at 5 mg/kg (method IL) and approximately 120-130 mg/kg (two results of method RS) (s. fig. 2).

Characteristics: Quantitative evaluation Soya (as Soyprotein)

Sample B

Characteristics	**All Results** *[mg/kg]*	**Methode RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	*9*	*8*
Number of outliers	*1*	*1*
Median	76,4	83,0
Robust mean (X_{pt})	85,0	94,2
Robust standard deviation (S*)	55,8	45,6
Target data:		
Target standard deviation σ_{pt}	21,2	23,6
lower limit of target range ($X_{pt} - 2\sigma_{pt}$)	42,5	47,1
upper limit of target range ($X_{pt} + 2\sigma_{pt}$)	127	141
Quotient S/σ_{pt}*	*2,6*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*23,3*	*20,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*1,1*	*0,86*
Number of results in target range	*6*	*7*
Percent in target range	*67%*	*88%*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments to the statistical characteristics:

The evaluation of all methods a slightly increased variability of results. The quotients S/σ_{pt} was clearly above 2,0. The comparability of results across the methods is limited.*
The evaluation of results from method RS showed an acceptable variability. The quotient S/σ_{pt} was slightly below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied method (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given.*

The robust means of the evaluations were 52% and 57%, which was approximately half of the spiking level of soya to sample B fulfilling the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Soya" p. 21).

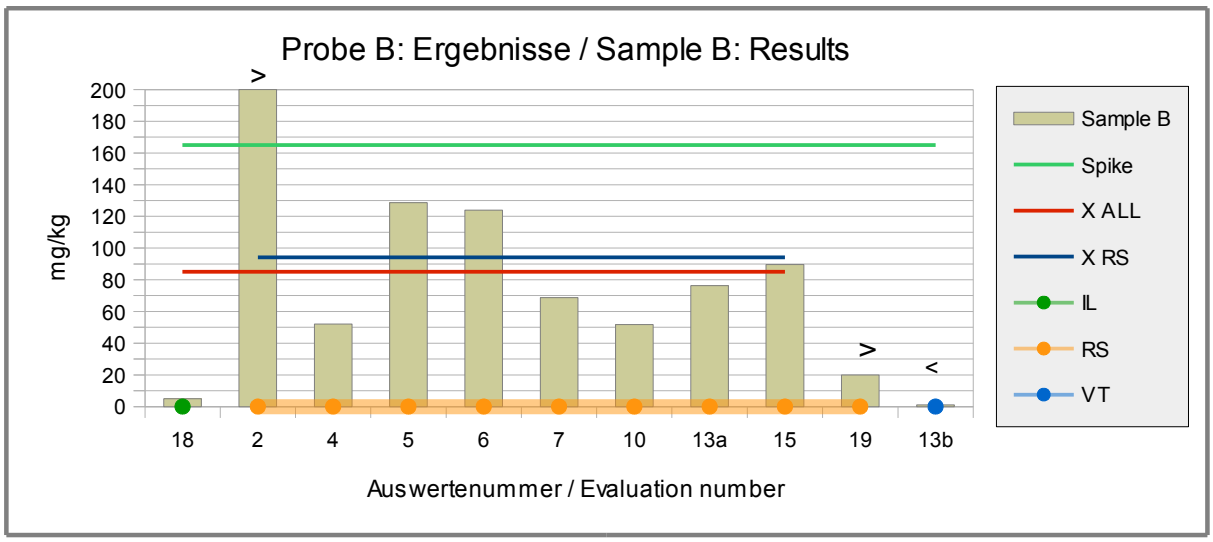

Fig. 3: ELISA-Results Soya (as Soyprotein)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS
round symbols = Applied methods (see legend)

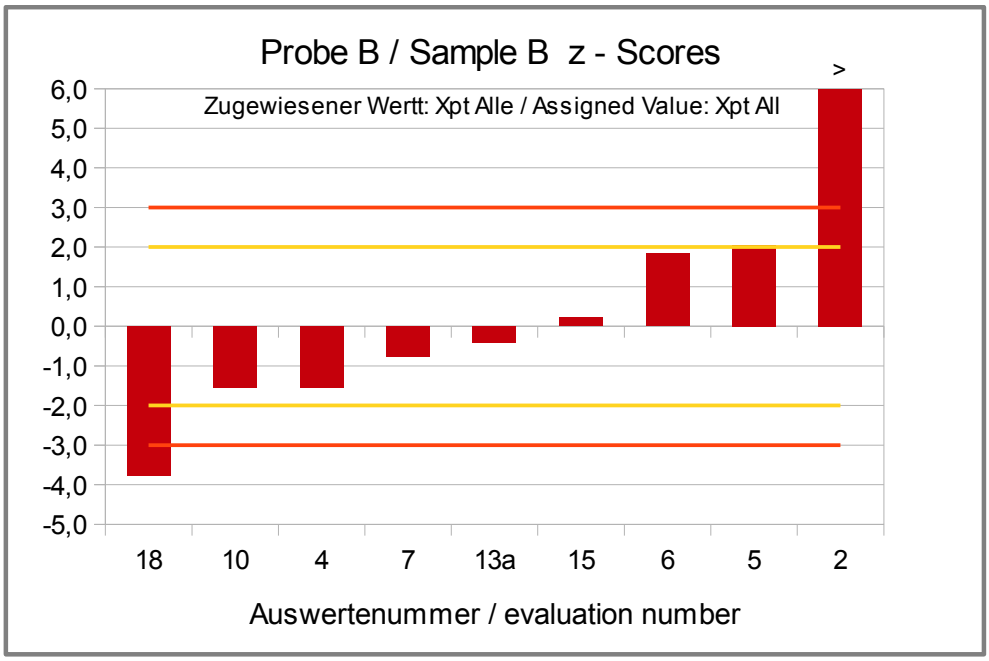

Fig. 4: z-Scores (ELISA-Results as Soyprotein)
Assigned value robust mean of all results

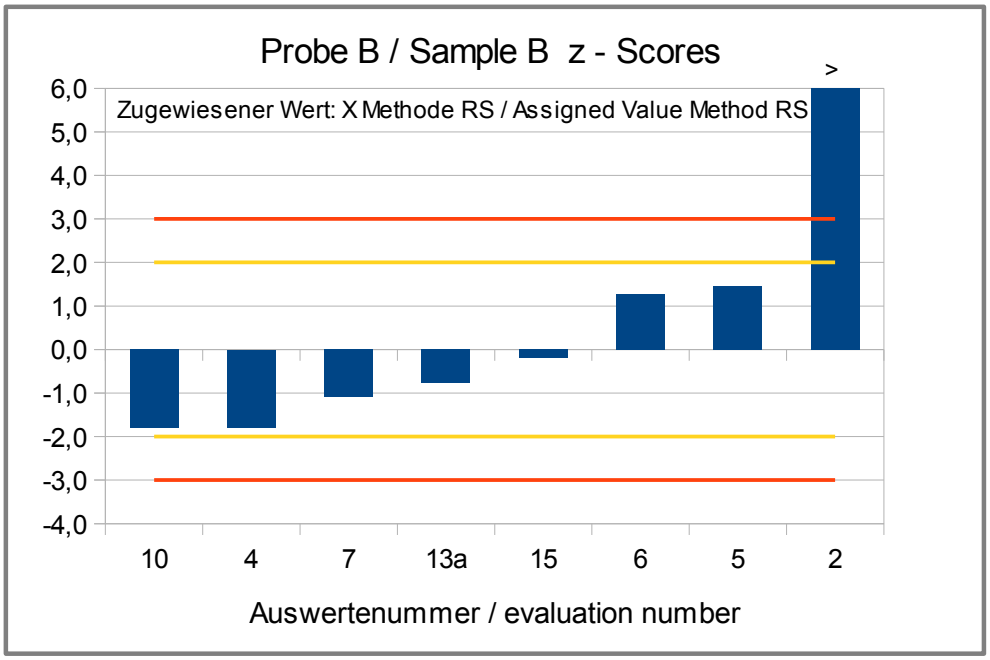

Fig. 5: z-Scores (ELISA-Results as Soyprotein)
Assigned value robust mean of method RS (R-Biopharm, Ridascreen)

Recovery Rates for Soya (as Soyprotein):
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
18	20000	249	5	3,0	IL	
2	>20		841	510	RS	
4	8530	**106**	52	32	RS	
5	5698	**71**	128,63	**78**	RS	
6			124	**75**	RS	
7	9860	**123**	68,7	42	RS	
10	6143,9	**76**	51,76	31	RS	
13a	5969	**74**	76,36	46	RS	
15			89,7	**54**	RS	
19	> 20		> 20		RS	
13b	157	2,0	< 1,0		VT	Result converted *

* calculation see p. 14

RA*	50-150 %	RA*	50-150 %
Number in RA	**5**	Number in RA	**3**
Percent in RA	**71**	Percent in RA	**33**

* Range of acceptance of AOAC for allergen ELISAS

Recovery rate
100% relative size:
Soyprotein, s. page 4

Methods:
IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT = Veratox, Neogen

Comments:
For the spiking material sample 71% of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the bread-sample B produced with the spiking material sample 33% of the recovery rates were in the range of acceptance.

4.1.2 PCR-Results: Soya

Evaluation number	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Qualitative Valuation	Method	Remarks
	pos / neg	mg/kg	pos / neg	mg/kg	Agreement with Consensus Value		
10	negative		positive		1/1 (100%)	ASU	
11	positive	< 10	positive	69	1/1 (100%)	ASU	
6	positive		positive		1/1 (100%)	SFA ID	
16a	positive	-	positive	-	1/1 (100%)	SFA ID	
3	positive	185	positive	6982	1/1 (100%)	SFA Quant	
14	positive		positive	152	1/1 (100%)	SFA Quant	
16b	positive	2,5	positive	114	1/1 (100%)	SFA Quant	
1	negative	-	positive	-	1/1 (100%)	div	
8	negative		positive	5000	1/1 (100%)	div	result given as Soya-DNA
9	negative		negative		0/1 (0%)	div	
19	negative		positive		1/1 (100%)	div	

	Sample A		Sample B	
Number positive	6		10	
Number negative	5		1	
Percent positive	55		91	
Percent negative	45		9	
Consensus value	none		positive	

Method:

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

Comments:

There were 91% positive results for sample B obtained by the PCR-methods. The consensus value is in agreement with the spiking of sample B.
For sample A there were 55% positive and 45% negative results. A consensus value (≥ 75%) could not be established for sample A. The positive results are most likely close to the limits of detection and quantification. The quantitative results of participants for sample A are indicating levels < 10 mg/kg (participant no. 3 obtained approximately 20 times increased results, see recovery rates p. 23).

Quantitative valuation of results: Sample B

There were < 5 quantitative results, therefore no statistical evaluation was done.

Recovery Rates for Soya (as Soybean / Soyflour): Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
10					ASU	
11	26000	**129**	69	17	ASU	
6					SFA ID	
16a	-		-		SFA ID	
3			6982	1691	SFA Quant	
14	11556	**57**	152	37	SFA Quant	
16b	12149	**60**	114	28	SFA Quant	
1	-		-		div	
8	17000	**85**	5000	1211	div	Recovery not valid, in case result given as Soya-DNA (s. documentation)
9					div	
19					div	

RA*	50-150 %	AB*	50-150 %
Number in RA	**4**	Anzahl im AB	**0**
Percent in RA	**100**	Prozent im AB	**0**

* Range of acceptance of AOAC for allergen ELISAS

Recovery rate
100% relative size:
Soy flour, see page 4

Methods:

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

Comments:

All four participants who submitted quantitative PCR-results for the spiking material sample obtained recovery rates within the range of acceptance of 50-150%. For the bread-sample B produced with the spiking material sample none of the recovery rates were in the range of acceptance. Three recovery rates were below 50% and two were about 12-17 times higher than 100%.

4.2 Proficiency Test 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		
12	negative	< 5	positive	< 5	2/2 (100%)	AQ	
18	negative	< 2	positive	4	2/2 (100%)	IL	Result converted *
1a	negative	< 5	positive	17	2/2 (100%)	RS1	
2	negative		positive	23	2/2 (100%)	RS1	
3	negative		positive	30,35	2/2 (100%)	RS1	
4	negative	< 10	positive	22	2/2 (100%)	RS1	Result converted *
5	negative	< 2,5	positive	24,6	2/2 (100%)	RS1	qualitative method: Lateral Flow (s. documentation)
6	negative		positive	20,4	2/2 (100%)	RS1	
7	negative	< 5,0	positive	18,9	2/2 (100%)	RS1	
9	negative	< 5	positive	20	2/2 (100%)	RS1	
10	negative	< 5	positive	22,64	2/2 (100%)	RS1	
11	negative	< 5	positive	16,2	2/2 (100%)	RS1	
13	negative	< 5	positive	16,83	2/2 (100%)	RS1	
14	negative	6	positive	27	2/2 (100%)	RS1	
15	negative	< 5	positive	16	2/2 (100%)	RS1	
19	negative	< 3	positive	16,7	2/2 (100%)	RS1	
17	positive	4,86	positive	9,73	1/2 (100%)	RS2	
1b	negative	< 10	positive	26	2/2 (100%)	VT	
8	negative		negative		1/2 (50%)	div	

* calculation see p. 14

	Sample A		Sample B	
Number positive	1		18	
Number negative	18		1	
Percent positive	5		95	
Percent negative	95		5	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
RS1 = Ridascreen®, R-Biopharm
RS2 = Ridascreen Fast®, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:

There were 95% negative results for sample A and 95% positive results for sample B by the ELISA-methods. One positive result was in the range of the LOQ of the method (evaluation no. 17, method RS2). One negative result was obtained by a in-house method (evaluation no. 8).
The consensus values are in agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

Evaluation number	Gluten	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	Bezug X_{ALL}	$X_{Methode\ RS}$		
12	< 5			AQ	
18	4	-3,2		IL	Result converted *
1a	17	-0,6	-0,7	RS1	
2	23	0,6	0,5	RS1	
3	30,35	2,1	1,9	RS1	
4	22	0,4	0,3	RS1	Result converted *
5	24,6	0,9	0,8	RS1	
6	20,4	0,1	0,0	RS1	
7	18,9	-0,2	-0,3	RS1	
9	20	0,0	-0,1	RS1	
10	22,64	0,6	0,4	RS1	
11	16,2	-0,7	-0,9	RS1	
13	16,83	-0,6	-0,7	RS1	
14	27	1,4	1,2	RS1	
15	16	-0,8	-0,9	RS1	
19	16,7	-0,6	-0,8	RS1	
17	9,73	-2,0		RS2	
1b	26	1,2		VT	
8				div	

* calculation see p. 14

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
RS1 = Ridascreen®, R-Biopharm
RS2 = Ridascreen Fast®, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

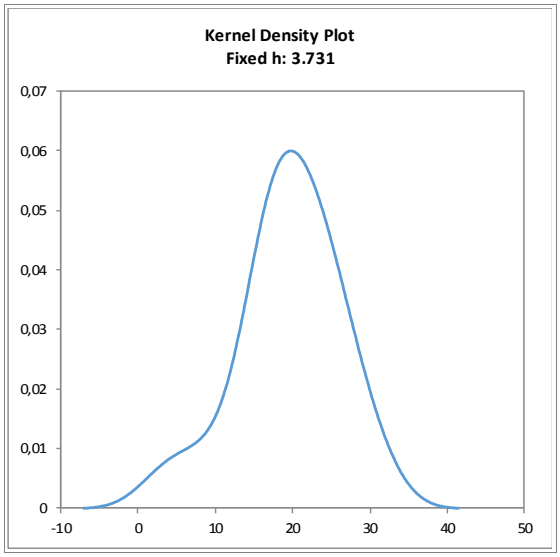

Fig. 6: Kernel Density Plot of all ELISA-results gluten
(with h = 0,75 x σpt of Xpt_{ALL})

Comment:
The kernel density estimation shows a normal distribution of results with a shoulder at 4 mg/kg (method IL) (s. fig. 6).

Characteristics: Quantitative evaluation Gluten

Sample B

Characteristics	**All Results** *[mg/kg]*	**Methode RS1** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	*17*	*14*
Number of outliers	*0*	*0*
Median	20,0	20,2
Robust mean (X_{pt})	19,9	20,6
Robust standard deviation (S*)	5,77	4,44
Target data:		
Target standard deviation σ_{pt}	4,98	5,15
lower limit of target range ($X_{pt} - 2\sigma_{pt}$)	9,95	10,3
upper limit of target range ($X_{pt} + 2\sigma_{pt}$)	29,9	30,9
Quotient S/σ_{pt}*	*1,2*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*1,75*	*1,48*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,35*	*0,29*
Number of results in target range	*15*	*14*
Percent in target range	*88%*	*100%*

Method:
RS1 = R-Biopharm, Ridascreen®

Comments to the statistical characteristics:

The evaluation of all methods and the evaluation of results from method RS1 showed a low variability, respectively. The quotients S/σ_{pt} were clearly below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there are only one result each for the methods IL, RS2 and VT.*

The robust means of the evaluations were with approximately 60% of the spiking level of soya to sample B within the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Gluten" p.29).

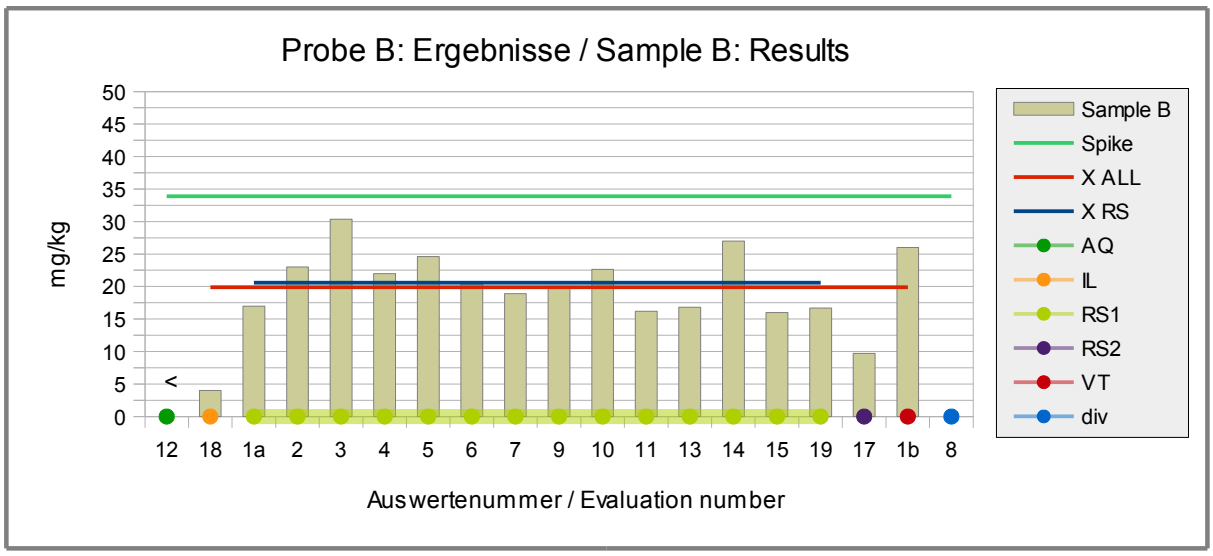

Fig. 7: ELISA-Results Gluten
green line = Spiking level (514 mg/kg, not indicated)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS1
round symbols = Applied methods (see legend)

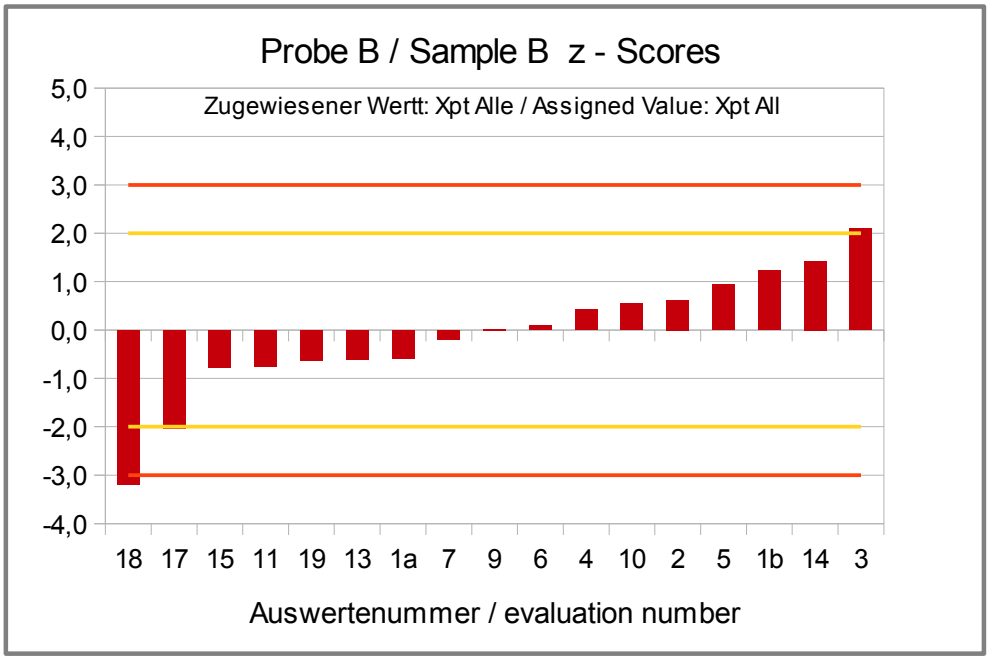

Fig. 8: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of all results

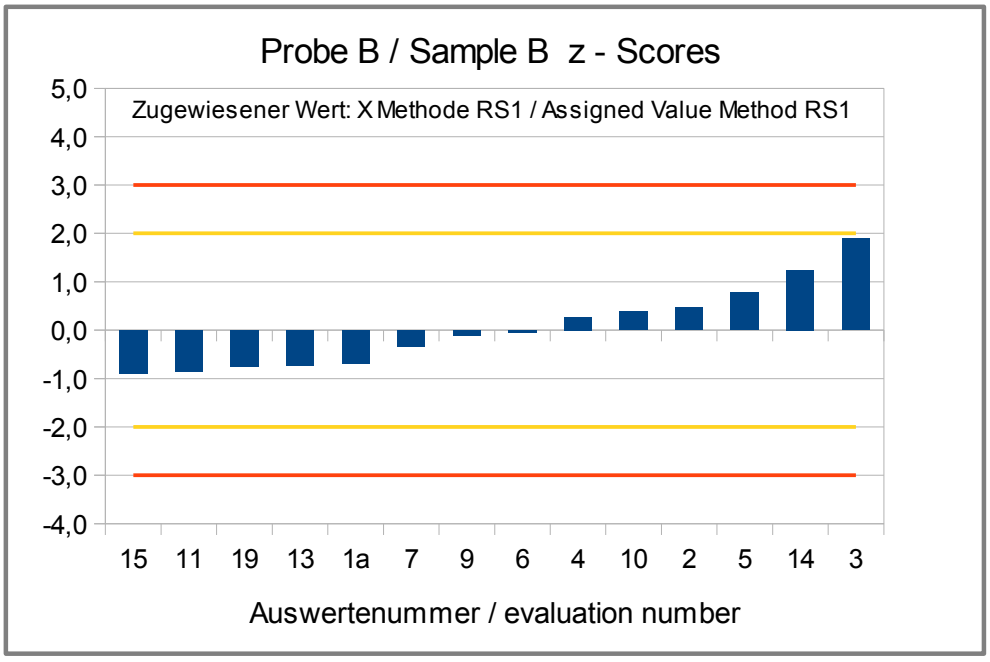

Fig. 9: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of method RS1
(R-Biopharm, Ridascreen)

Recovery Rates for Gluten:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
12	> 20		< 5		AQ	
18	4000	242	4	12	IL	Result converted *
1a	740	45	17	**50**	RS1	
2	500	30	23	**68**	RS1	
3			30,35	**90**	RS1	
4	774	47	22	**65**	RS1	Result converted *
5	> 80		24,6	**73**	RS1	
6			20,4	**60**	RS1	
7	2164	**131**	18,9	**56**	RS1	
9	-		20	**59**	RS1	
10	701,5	43	22,64	**67**	RS1	
11	560	34	16,2	48	RS1	
13	673	41	16,83	**50**	RS1	
14	1000	**61**	27	**80**	RS1	
15			16	47	RS1	
19	> 80		16,7	49	RS1	
17	339,71	21	9,73	29	RS2	
1b	1100	**67**	26	**77**	VT	
8	77,5	5			div	

* calculation see p. 14

RA*	50-150 %	RA*	50-150 %
Number in RA	**3**	Number in RA	**11**
Percent in RA	**25**	Percent in RA	**65**

* Range of acceptance of AOAC for allergen ELISAS

Recovery rate
100% relative size:
Gluten, s. page 4

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
RS1 = Ridascreen®, R-Biopharm
RS2 = Ridascreen Fast®, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:
For the spiking material sample 25% of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the baked bread-sample B produced with the spiking material sample 65% of the recovery rates were in the range of acceptance.

4.2.2 PCR-Results: Wheat

Evaluation number	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Qualitative Valuation	Method	Remarks
	pos / neg	mg/kg	pos / neg	mg/kg	Agreement with Consensus Value		
6	traces		positive		1/1 (100%)	SFA ID	
19a	positive		positive		1/1 (100%)	SFA ID	
16	positive	< 1	positive	5,3	1/1 (100%)	SFA Quant	result as gluten containing cereals
1	negative	-	positive	-	1/1 (100%)	div	
8	negative		negative	0	0/1 (0%)	div	result given as Wheat-DNA
9	negative		negative		0/1 (0%)	div	
10	-		-		0/1 (0%)	div	
11	negative	< 80	positive	< 200	1/1 (100%)	div	result given as w heat
19b	negative		positive		1/1 (100%)	div	

	Sample A		Sample B	
Number positive	2		6	
Number negative	5		2	
Percent positive	29		75	
Percent negative	71		25	
Consensus value	none		positive	

Methods:

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

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

div = not indicated / other method

Comments:

There were 75% positive results for sample B for wheat-DNA by the PCR-methods. The consensus value is therefore in agreement with the spiking of sample B.

For sample A there were 29% positive and 71% negative results as well as one result indicating "traces". A consensus value (≥ 75%) could not be established for sample A.

Quantitative valuation of results: Sample B

There were < 5 quantitative results, therefore no statistical evaluation was done.

Recovery Rates for Wheat:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
6					SFA ID	
19a					SFA ID	
16	3041	20	5,3	1,7	SFA Quant	result as gluten containing cereals
1	-		-		div	
8	50000	327	0	0	div	Recovery not valid, in case result given as Wheat-DNA (s. documentation)
9					div	
10					div	
11	73400	480	< 200		div	result given as w heat
19b					div	

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

* Range of acceptance of AOAC for allergen ELISAS

Recovery rate
100% relative size:
Wheat flour,, s. page 4

Methods:

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

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

div = not indicated / other method

Comments:

Three participants reported quantitative PCR-results. None of the recovery rates for the spiking material sample as well as for the baked bread-sample B produced with the spiking material were within the range of acceptance of 50-150%.

5. Documentation

Details by the participants

5.1 ELISA: Soya

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
18	negative	< 1	positive	5	positive	20000	Soyprotein	IL	Immunolab Soy ELISA
2	negative		positive	841	positive	>20	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
4	negative	< 4	positive	52	positive	8530	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
5	negative	< 2,5	positive	128,63	positive	5698	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
6	negative		positive	124	positive		Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
7	negative	< 2,5	positive	68,7	positive	9860	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
10	negative	< 10	positive	51,76	positive	6143,9	given as Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
13a	negative	< 2.5	positive	76,36	positive	5969	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
15	-	< 2,5	-	89,7	-		Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
19	negative	< 1,25	positive	> 20	positive	> 20		RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
13b	negative	< 2,5	positive	< 2,5	positive	392	Soy flour	VT	Veratox Soy, Neogen

Methods:

IL = Immunolab
RS = Ridascreen®, R-Biopharm
VT = Veratox, Neogen

Other details to the Methods

Evaluation number	Meth. Abk.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
18	IL	STI		
2	RS	Soya protein	R-biopharm method version 13-12-10	
4	RS	antisoya protein		
5	RS		sample preparation and testing according to testkit instructions	
6	RS		As Per Kit Instructions	
7	RS		As Per Kit Instructions	
10	RS		As Per Kit Instructions	
13a	RS	Heat Treated Soy Proteins	As Per Kit Instructions	
15	RS		10min / 100ºC	
19	RS			
13b	VT	Soya Proteins	As Per Kit Instructions	

5.2 ELISA: Gluten

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
12	negative	< 5	negative	< 5	positive	> 20	Gluten	AQ	AgraQuant Gluten (COKAL0200), RomerLabs
18	negative	< 1	positive	2 gl	positive	2000 gl	Gliadin	IL	Immunolab Gliadin GLU-E02
1a	negative	< 5	positive	17	positive	740	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
2	negative		positive	23	positive	500	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
3	negative		positive	30,35	positive		Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
4	negative	< 5	positive	11 gl	positive	387 gl	Gliadin	RS1	Ridascreen Gluten (R7001), r-Biopharm
5	negative	< 2,5	positive	24,6	positive	> 80	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
6	negative		positive	20,4	positive		Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
7	negative	< 5,0	positive	18,9	positive	2164	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
9	negative	< 5	positive	20	-	-		RS1	Ridascreen Gluten (R7001), r-Biopharm
10	negative	< 5	positive	22,64	positive	701,5	given as Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
11	negative	< 5	positive	16,2	positive	560	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
13	negative	< 5	positive	16,83	positive	673	Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
14	positive	6	positive	27	positive	1000		RS1	Ridascreen Gluten (R7001), r-Biopharm
15	-	< 5	-	16	-		Gluten	RS1	Ridascreen Gluten (R7001), r-Biopharm
19	negative	< 3	positive	16,7	positive	> 80		RS1	Ridascreen Gluten (R7001), r-Biopharm
17	-	4,86	-	9,73	positive	339,71		RS2	Ridascreen Fast Gluten (R7002), r-Biopharm
1b	negative	< 10	positive	26	positive	1100	Gluten	VT	Veratox Gliadin, Neogen
8	negative		negative		positive	77,5	Gluten-Protein	div	in house

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
RS1 = Ridascreen®, R-Biopharm
RS2 = Ridascreen Fast®, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Other details to the methods

Evaluation number	Meth. Abr.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	
12	AQ			
18	IL	Gliadin		
1a	RS1			
2	RS1	R5	R-Biopharm method version 12-04-18	
3	RS1	r5	esxtracted with C.solution Mendez	
4	RS1	antigliadin		
5	RS1		sample preparation and testing according to testkit instructions. Using cocktail solution R7006	samples A and B tested with RIDA®QUICK Gliadin R7004 (qualitative)
6	RS1		As Per Kit Instructions	
7	RS1		As Per Kit Instructions	
9	RS1	R5	according to manual	
10	RS1		As Per Kit Instructions	
11	RS1	Gliadin	cocktail extraction solution	
13	RS1	Monoclonal R5 antibody	As Per Kit Instructions	Mendez R5 Method
14	RS1			
15	RS1	R5-Mendez	Solución cokctail/40min/50ºC+ Etanol80%/1 hora/Tªambiente	
19	RS1			
17	RS2	Gliadin	RIDA Extraction Solution R7009/2 hours/ 60°C water bath	
1b	VT			
8	div			

5.3 PCR: Soya

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
10	negative		positive		positive		DNA-Soya	ASU	ASU § 64 LFGB L 00.00-105, annex C.2 (modified)
11	positive	< 10	positive	69	positive	26000	Soyflour	ASU	ASU L 08.00-59 : 2013-01
6	positive		positive		positive		DNA-Soya	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
16a	positive	-	positive	-	positive	-	Soybean, total	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
3	positive	185	positive	6982	positive		Soybean	SFA Quant	Sure Food Allergen QUANT, Congen / r-Biopharm
14	positive		positive	152	positive	11556		SFA Quant	Sure Food Allergen QUANT, Congen / r-Biopharm
16b	positive	2,5	positive	114	positive	12149	Soybean, total	SFA Quant	Sure Food Allergen QUANT, Congen / r-Biopharm
1	negative	-	positive	-	positive	-	DNA-Soya	div	internal method
8	negative		positive	5000	positive	17000	"Soya-DNA"	div	in house
9	negative		negative		positive		Soy DNA	div	in house method
19	negative		positive		positive		DNA-Soya	div	Internal method

Method:

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

Other Remarks to the Methods

Evaluation number	Meth. Abr.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
10	ASU	Lectin Gene (74 bp)	according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride-buffer with Proteinase K, clean-up by Wizard-Kit from Promega) Real-time PCR with 45 cycles	sample B: < 120 haploid genomic copies; spiking material sample: < 60.500 haploid genomic copies
11	ASU	Lectin-Gen, 81 bp		
6	SFA ID		As per ki instructions	
16a	SFA ID	-	S3401 SureFood®ALLERGEN 4plex Soya/Celery/Mustard+IAC LOD 0,4 mg/kg Extraction with S1053 SureFood® PREP Advanced, Protocol 1	-
3	SFA Quant		extract with preo advanced r-biopharm, RT-PCR, 45cycles	
14	SFA Quant			sample A: only traces detectable, between LOD and LOQ; quantitative indication not allowed
16b	SFA Quant	-	S3201 SureFood®ALLERGEN QUANT Soya LOD 0,4 mg/kg Extraction with S1053 SureFood® PREP Advanced, Protocol 1	-
1	div		CTAB / Protease K / Chloroform + Promega Wizard/ Realtime PCR/ - / 45 Zyklen	
8	div	lectin	Wizard	
9	div			
19	div			

5.4 PCR: Wheat

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
6	Spuren		positive		positive		DNA-gluten containing cereals	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
19a	positive		positive		positive		Gluten	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
16	positive	< 1	positive	5,3	positive	3041	other: gluten containing cereals	SFA Quant	Sure Food Allergen QUANT, Congen / r-Biopharm
1	negative	-	positive	-	positive	-	Wheat, barley, rye DNA (indirectly Gluten)	div	internal method
8	negative		negative	0	positive	50000	"Wheat-DNA"	div	in house
9	negative		negative		positive		Wheat DNA	div	in house method
10	-		-		positive		DNA-Wheat/Spelt	div	nach Iida et al.(2005) J Agric. Food Chem 53: 6294-6300
11	negative	< 80	positive	< 200	positive	73400	Wheat	div	ASU, in preparation (2016)
19b	negative		positive		positive		DNA-Wheat	div	Internal method

Methods:

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

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

div = not indicated / other method

Other Remarks to the Methods

Evaluation number	Meth. Abr.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
6	SFA ID		As per kit instructions	
19a	SFA ID			
16	SFA Quant	-	S3206 SureFood®ALLERGEN QUANT Gluten LOD 0,4 mg/kg LOQ 1 mg/kg Extraction with S1053 SureFood® PREP Advanced, Protocol 1	sample A was positive below the LOQ tested (content > 0,4 mg/kg and < 1 mg/kg)
1	div		CTAB / Protease K / Chloroforme + Promega Wizard/ End point PCR/ 4% Agarose gel / 45 cycles	
8	div	Wheat	Wizard	
9	div			
10	div	waxy-D1 Gene (102 bp)	according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride-buffer with Proteinase K, clean-up by Wizard-Kit from Promega) Real-time PCR with 45 cycles	scope of PCR-detection not suitable for allergen detection of gluten, therefore result given only for spiking material sample and not for sample A and B; spiking material sample: < 2.400 haploid genomic copies
11	div	HMW Glutenin Gene B1-1 of Wheat and 1-R of Rye, 85 bp		
19b	div			

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		Germany
		FRANCE
		Germany
		Germany
		Germany
		Germany
		Germany
		FRANCE
		Germany
		FRANCE
		Germany
		ITALY
		Germany
		SWITZERLAND
		BELGIUM
		Germany
		UNITED KINGDOM
		SWEDEN
		UNITED KINGDOM

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

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

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