DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

02/2014

Allergens II:

Soya and Wheat

in "gluten-free" Pastry

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Inhalt

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 validity of the particular testing method.
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.

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 materials are common in commerce baking mixture labeled "gluten free". The basic composition of both sample A and sample B was the same (see table 1). The spiking material sample containing soy flour and wheat flour was added to sample B. 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**
Baking mixture "Marble Cake", glutenfree Ingredients: Baking mixture (93%) - corn starch, sugar, corn flour, rice flour, humectant: sorbitol, acidifier: biphosphates, baking agent: sodium hydrogencarbonate, thickener: guar gum, aroma, salt; cacao mixture (7%) - sugar, defatted cacao powder	100 g/100g	99,2 g/100g

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Sample B**
Potato flour	83 %	0,80 %
Soya: - as Soy flour - thereof Soyproteins	 62100 mg/kg (6,21 %) 24840 mg/kg	 497 mg/kg 199 mg/kg
Whole egg powder	3,41 %	0,027 %
Skimmed milk powder	4,84 %	0,039 %
Wheat: - as Wheat flour Typ 550 - thereof total protein - *thereof gluten***	 26500 mg/kg (2,65 %) 2809 mg/kg 2528 mg/kg	 212 mg/kg 22,5 mg/kg 20,3 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 spiked sample B was checked by 5fold ELISA-test. The resulting standard deviation between the samples of < 15% ensured sufficient homogeneity (17, 18, 20).

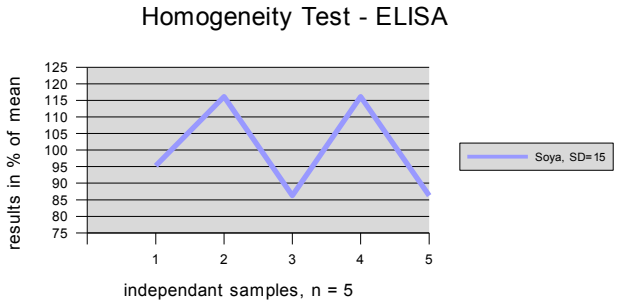

Fig. 1: Testing of homogeneity of DLA-sample B
Results are given in percent of the arithmetic mean

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 12th week of 2014. The testing method was optional. The tests should be finished at May 5th 2014 the latest.

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out along with the samples. On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. soyprotein or 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.
All 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. 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 actually added amount, in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method.

For ELISA-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 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6). In case the submitted results show hints for bimodal distribution or other reasons for a higher variability the evaluation will be performed additionally with respect to the robust mean of single methods. 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_{ALL}***
ii) ***Robust mean of single methods - $X_{METHOD\ i}$***
with at least 5 quantitative results given.

Single results giving values outside the measuring range of the participating laboratory were considered for statistical evaluation (e.g. results given as > 25 mg/kg and < 2,5 mg/kg, respectively) when a result indicating „>" is above and a result indicating „<" is below the target range.

3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S^x) was calculated (6).
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 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic for 95% significance niveau (5). Detected outliers were stated for information only, when z-score was < -2 or > 2.

3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is derived from following equation (Horwitz)

$$\hat{\sigma}_{(\%)} = 2^{(1-0,5\log X)}$$

From the result the target standard deviation is calculated

$$\hat{\sigma} = X * \hat{\sigma}_{(\%)} / 100.$$

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 the between-laboratories standard deviation can be calculated σ_L :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \quad .$$

And then, using the number of replicate measurements n, each participant is to perform, the target standard deviation for proficiency assessment is calculated :

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

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 $\hat{\sigma}$.

The following table shows the relative reproducibility standard deviations from proficiency tests of ELISA-methods from German ASU §64 methods (13, 14, 15):

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%.

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA-test kits for the quantification of peanut (16). 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%.

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 (21). 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 (21).

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 (6).
Criteria for the level of performance of analytical methods for the quantitative determination of allergens in foods with ELISA were recently elaborated by the Ministry of Health and Welfare (MHLW) in Japan (17), by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 (18, 19) and by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens (20).

Some of the relevant ELISA validation criteria of the three panels are listed below:

Literature (17, 18, 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)]

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

Based on the currently achievable level of performance of ELISA 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 $\hat{\sigma}$ 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 ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X) (6).
Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \ ;$$

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)*

3.6 Quotient $S^x/\hat{\sigma}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation 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 (11).

3.7 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty u_X for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x / \sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT (6). The Quotient $u_X/\hat{\sigma}$ is reported in the characteristics of the test.

3.8 Figures

The assigned values 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 (20).

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.

ELISA-results, which were given as soya flour or gliadin, were converted into soya proteins and gluten with respect to the instructions of the test kit manufacturers. The original results are given in the documentation.

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_{Mi}	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:

	All Results *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ i}$
Number of results		
Robust mean (X)		
Robust standard deviation (S^x)		
Median		
Target range:		
Target standard deviation ($\hat{\sigma}$)		
lower limit of target range (X - 2 $\hat{\sigma}$)		
upper limit of target range (X + 2 $\hat{\sigma}$)		
Quotient $S^x/\hat{\sigma}$		
Standard uncertainty u_X		
Quotient $u_X/\hat{\sigma}$		
Number of results in the 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		
3	negative	<0,27	positive	4,1	2/2 (100%)	AQ	
4	negative	<2,5	positive	12	2/2 (100%)	ES	
5	negative	< 2,5	positive	12	2/2 (100%)	ES	
6	negative	<1,25	positive	9,4	2/2 (100%)	ES	
26	negative		positive	5,85	2/2 (100%)	ES	
24	negative		positive	164	2/2 (100%)	IL	Result converted
1	negative	<2,5	positive	216,58	2/2 (100%)	RS	
9	negative	<2,5	positive	103	2/2 (100%)	RS	
11	negative	<2,5	positive	194,8	2/2 (100%)	RS	
16	negative	<2,5	positive	206	2/2 (100%)	RS	Result converted
17	negative	<2,5	positive	168,62	2/2 (100%)	RS	
18	negative	< 5.00	positive	138,04	2/2 (100%)	RS	
20	negative	< NWG	positive	209	2/2 (100%)	RS	
22	negative	<2,5	positive	104	2/2 (100%)	RS	
28	negative	<NG	positive	>20	2/2 (100%)	RS	
7	negative	<1,0	positive	7,8	2/2 (100%)	VT	Result converted
10	negative		positive		2/2 (100%)	VT	
13	negative	<1,0	positive	10,4	2/2 (100%)	VT	Result converted
19	negative	<1,0	positive	11,2	2/2 (100%)	VT	Result converted

	Sample A		Sample B	
Number positive	0		19	
Number negative	19		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus	negative		positive	

Method:

AQ = AgraQuant, RomerLabs
ES = ELISA Systems
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Comments:

There were 100% negative results for sample A and 100% positive results for sample B by the ELISA-methods. The results are in qualitative agreement with the spiking of sample B.

Quantitative evaluation of results: Sample B

Evaluation number	Soy Protein	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
3	4,1			AQ	
4	12			ES	
5	12			ES	
6	9,4			ES	
26	5,85			ES	
24	164			IL	Result converted
1	216,58		1,2	RS	
9	103		-1,5	RS	
11	194,8		0,6	RS	
16	206		0,9	RS	Result converted
17	168,62		0,0	RS	
18	138,04		-0,7	RS	
20	209		1,0	RS	
22	104		-1,5	RS	
28	>20			RS	
7	7,8			VT	Result converted
10				VT	
13	10,4			VT	Result converted
19	11,2			VT	Result converted

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA Systems
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Characteristics: Quantitative evaluation Soya (as Soy Protein)

Sample B

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*17*	*8*
Robust mean (X)	92,8	168
Robust standard deviation (S^x)	98,7	53,2
Median	103	182
Target range:		
Target standard deviation ($\hat{\sigma}$)		42,0
lower limit of target range (X - 2 $\hat{\sigma}$)		84,0
upper limit of target range (X + 2 $\hat{\sigma}$)		252
Quotient $S^x/\hat{\sigma}$		*1,3*
Standard uncertainty u_X		*23,5*
Quotient $u_X/\hat{\sigma}$		*0,56*
Number of results in the target range		*8 (100%)*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments:
The evaluation of all methods showed a bimodal distribution of the results in the range of and of 4-12 mg/kg and 103-217 mg/kg. Statistical evaluation of all methods together was therefor not possible. The robust mean and standard deviation are given for information only.

The evaluation of results from method RS showed a low variability. The quotient $S^x/\hat{\sigma}$ was clearly below 2,0.
The mean of the evaluation was about 84% of the spiking level (s. "Recovery rates of Soy Protein" p.17).

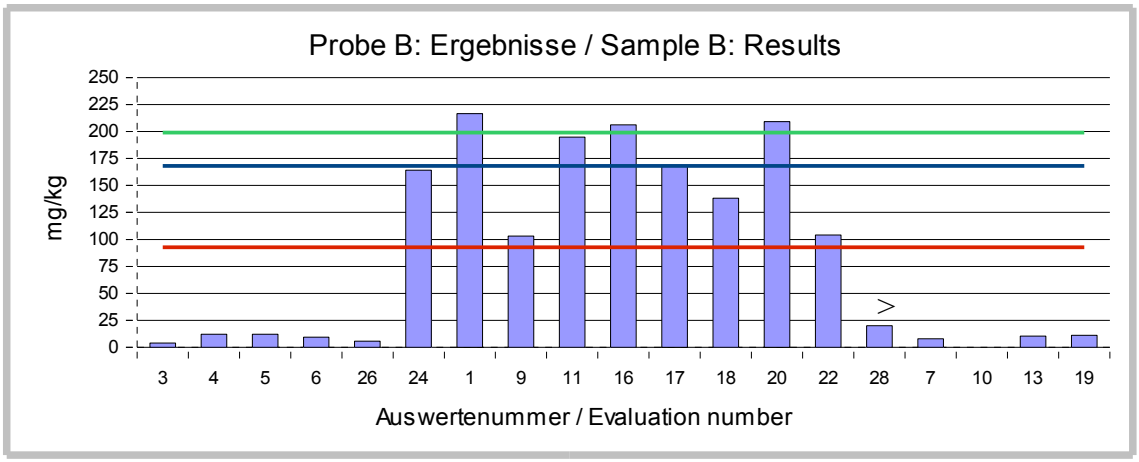

Fig. 2: ELISA-Results Soy (as Soy Protein)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

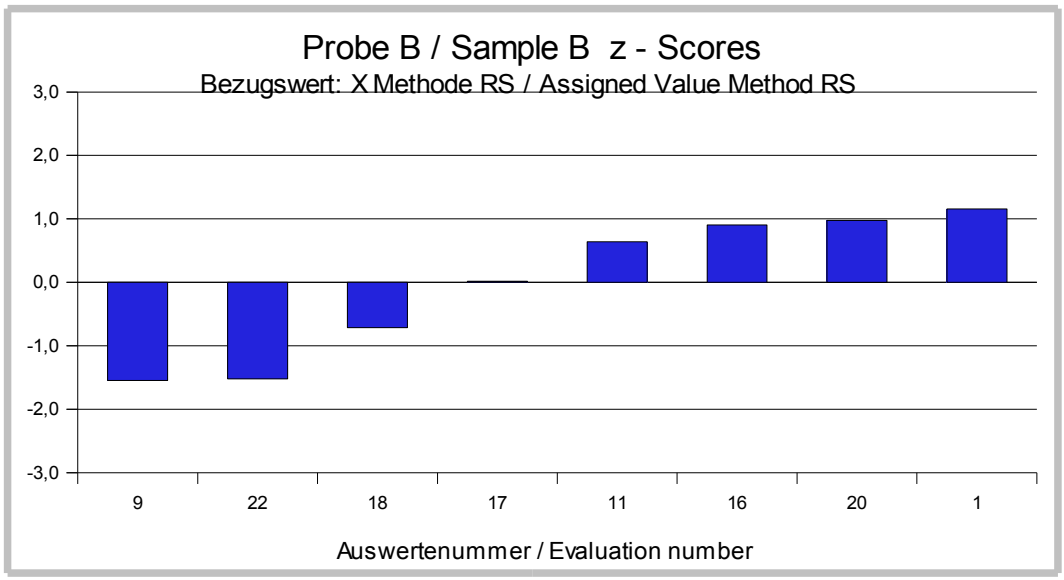

Fig. 3: z-Scores (ELISA-Results as Soy Protein) Assigned value robust mean of method RS (R-Biopharm, Ridascreen Fast)

Recovery Rates for Soy Protein:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3	521,1	2	4,1	2	AQ	
4	3025	12	12	6	ES	
5	3400	14	12	6	ES	
6	>25		9,4	5	ES	
26			5,85	3	ES	
24	18800	**76**	164	**82**	IL	Result converted
1	32200	**130**	216,58	**109**	RS	
9	9047	36	103	**52**	RS	
11	29989	**121**	194,8	**98**	RS	
16	29730	**120**	206	**104**	RS	Result converted
17	26738,94	**108**	168,62	**85**	RS	
18	3400	14	138,04	**69**	RS	
20	18166	**73**	209	**105**	RS	
22	29714	**120**	104	**52**	RS	
28	>20		>20		RS	
7	1360	5	7,8	4	VT	Result converted
10					VT	
13	>1000		10,4	5	VT	Result converted
19	2000	8	11,2	6	VT	Result converted

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

* Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA Systems
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Comments:

For the spiking material sample 7 participants obtained recovery rates within the range of the AOAC-recommendation of 50-150%. For the backing mixture-sample B produced with the spiking material sample 9 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		
14	negative		positive		2/2 (100%)	ASU-1	
23	negative		positive		2/2 (100%)	ASU-1	
15	negative		positive	438	2/2 (100%)	ASU-2	
5	-		positive	> 5	1/2 (50%)	SFA	
16	positive	2,1	positive	231	1/2 (50%)	SFA	
21	positive	21,7	positive	> 400	1/2 (50%)	SFA	
2	negative		positive	1333	2/2 (100%)	div	
6	negative	-	positive	+	2/2 (100%)	div	
8	negative		positive	1400	2/2 (100%)	div	
12	positive		positive		1/2 (50%)	div	
13	negative		positive		2/2 (100%)	div	
19	negative	-	positive	-	2/2 (100%)	div	
25	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	3		13	
Number negative	9		0	
Percent positive	25		100	
Percent negative	75		0	
Konsenswert	negative		positive	

Methods:

ASU-1 = ASU L 00.00-105, ASU-2 = ASU L 08.00-59
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:

For the detection of soya by PCR 75% negative results for sample A and 100% positive results for sample B were obtained.
The quantitative results were given with differing relations, as "DNA-Soya", "Soybean" or "Soyflour".

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]	[%]		
14					ASU-1	
23					ASU-1	
15			438	**88**	ASU-2	
5	> 5		> 5		SFA	
16	55859	**90**	231	46	SFA	
21			> 400		SFA	
2	323500	521	1333	268	div	as DNA ???
6					div	
8	170000	274	1400	281	div	
12					div	
13					div	
19	-				div	
25					div	

RA*	50-150 %	RA*	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	33	Percent in RA	25

* Range of acceptance of AOAC for allergen ELISAs

Methods:
ASU-1 = ASU L 00.00-105, ASU-2 = ASU L 08.00-59
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:
One participant obtained a recovery rate in the range of 50-150% using PCR. For the baking mixture sample B spiked with the spiking material sample one of the recovery rates was in the range of acceptance too.

4.2 Proficiency Test Wheat

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		
16	negative	<4	positive	36	2/2 (100%)	AQ	
28	positive	6,8	positive	39,14	1/2 (50%)	AQ	
10	negative		positive	9	2/2 (100%)	BK	
24	negative		positive	6	2/2 (100%)	IL	
1	negative	<5	positive	23,05	2/2 (100%)	RS	
2	negative		positive	30	2/2 (100%)	RS	
3	negative	<5,0	positive	28	2/2 (100%)	RS	
4	negative	<5,0	positive	32	2/2 (100%)	RS	
5	negative	< 3	positive	33	2/2 (100%)	RS	
6	negative	<3	positive	31,7	2/2 (100%)	RS	
7	negative	<5	positive	25,65	2/2 (100%)	RS	
9	negative	<5,0	positive	28,7	2/2 (100%)	RS	
11	negative	<5,0	positive	34,1	2/2 (100%)	RS	
14	negative		positive	30	2/2 (100%)	RS	
15	-		positive	33	1/2 (50%)	RS	
16	positive	6,3	positive	34	1/2 (50%)	RS	
17	negative	<5,0	positive	26,06	2/2 (100%)	RS	
18	negative	< 5.00	positive	25,46	2/2 (100%)	RS	
19	negative	<5	positive	38	2/2 (100%)	RS	
20	negative	< NWG	positive	29	2/2 (100%)	RS	
23	negative	<5	positive	30,5	2/2 (100%)	RS	
26	negative		positive	25,5	2/2 (100%)	RS	
27	negative	<3	positive	15	2/2 (100%)	RS	
13	negative	<5	positive	25	2/2 (100%)	VT	
8	negative		negative		1/2 (50%)	div	

	Sample A		Sample B	
Number positivee	2		24	
Number negativee	22		1	
Percent positivee	8		96	
Percent negativee	92		4	
Consensus	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen
div = not indicated / other method

Comments:
There were 92% negative results for sample A (with 2 positive results in the range of the LOQ) and 96% positive results for sample B (with one negative result) by the ELISA-methods. The results are in qualitative 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]	X_{ALL}	$X_{Method\ RS}$		
16	36	1,0	0,9	AQ	
28	39,14	1,4	1,3	AQ	
10	9	-2,8	-2,8	BK	Outlier X_{All}
24	6	-3,2	-3,2	IL	Outlier X_{All}
1	23,05	-0,8	-0,9	RS	
2	30	0,1	0,1	RS	
3	28	-0,1	-0,2	RS	
4	32	0,4	0,4	RS	
5	33	0,6	0,5	RS	
6	31,7	0,4	0,3	RS	
7	25,65	-0,5	-0,5	RS	
9	28,7	0,0	-0,1	RS	
11	34,1	0,7	0,6	RS	
14	30	0,1	0,1	RS	
15	33	0,6	0,5	RS	
16	34	0,7	0,6	RS	
17	26,06	-0,4	-0,5	RS	
18	25,46	-0,5	-0,5	RS	
19	38	1,2	1,2	RS	
20	29	0,0	-0,1	RS	
23	30,5	0,2	0,1	RS	
26	25,5	-0,5	-0,5	RS	
27	15	-1,9	-2,0	RS	
13	25	-0,6	-0,6	VT	
8				div	

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen
div = not indicated / other method

Characteristics: Quantitative evaluation Gluten

Sample B

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*24*	*19*
Robust mean (X)	29,0	29,4
Robust standard deviation (S^x)	6,1	4,4
Median	29,5	30,0
Target range:		
Target standard deviation ($\hat{\sigma}$)	7,25	7,35
lower limit of target range (X - 2 $\hat{\sigma}$)	14,5	14,7
upper limit of target range (X + 2 $\hat{\sigma}$)	43,5	44,1
Quotient $S^x/\hat{\sigma}$	*0,84*	*0,60*
Standard uncertainty u_X	*1,56*	*1,26*
Quotient $u_X/\hat{\sigma}$	*0,21*	*0,17*
Number of results in the target range	*22 (92%)*	*19 (100%)*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments:
The evaluation of all methods and of method RS showed low variabilities. The quotients $S^x/\hat{\sigma}$ were below 1,0. Therefore the comparability was fair. The mean of the evaluations of all results and of method RS were about one third higher than the spiking level (s. also "Recovery rates of Gluten" p.25). It should be noted, that a gluten-content of the basic matrix "baking mixture" could not be excluded (sample A: 92% of results < 5 mg/kg)).

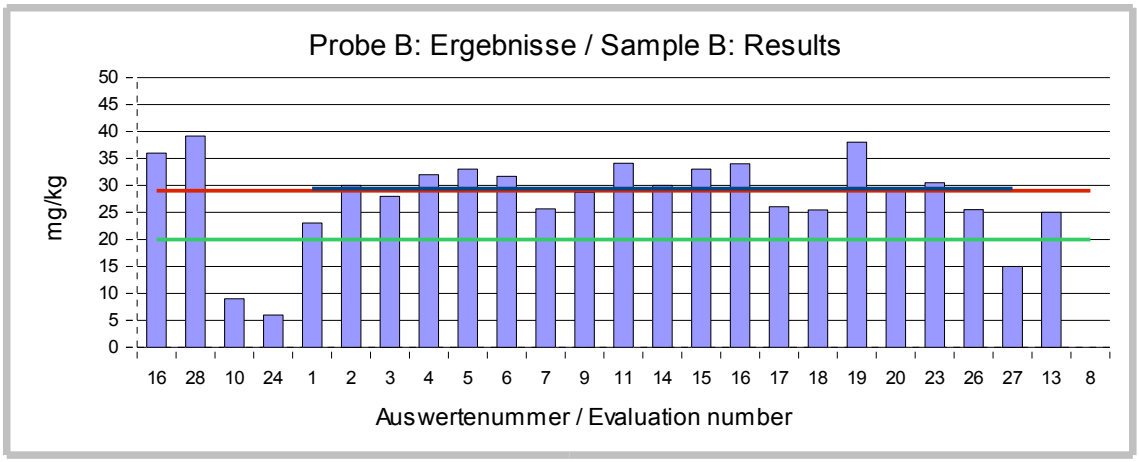

Fig. 5: ELISA-Results Gluten
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

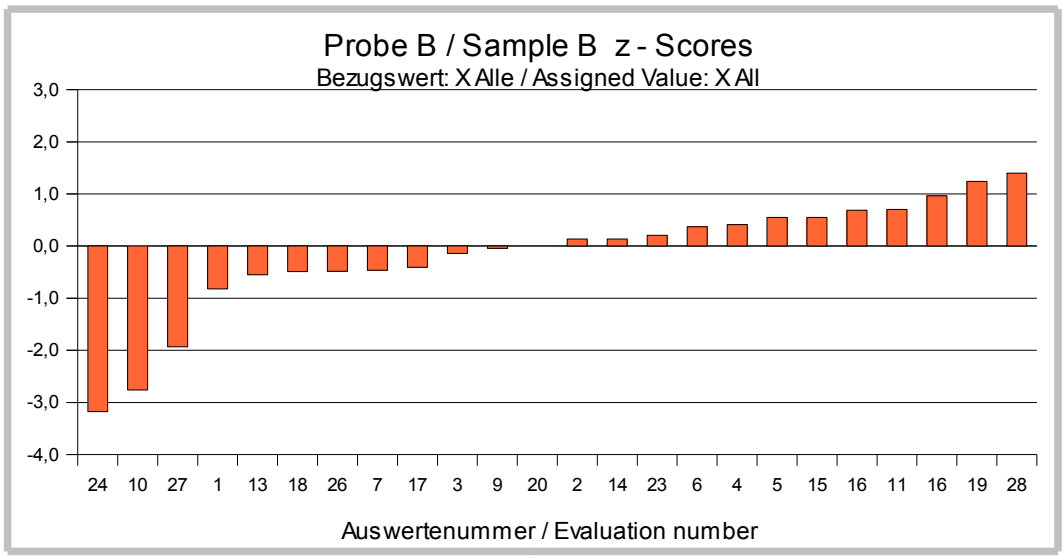

Fig. 6: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of all results

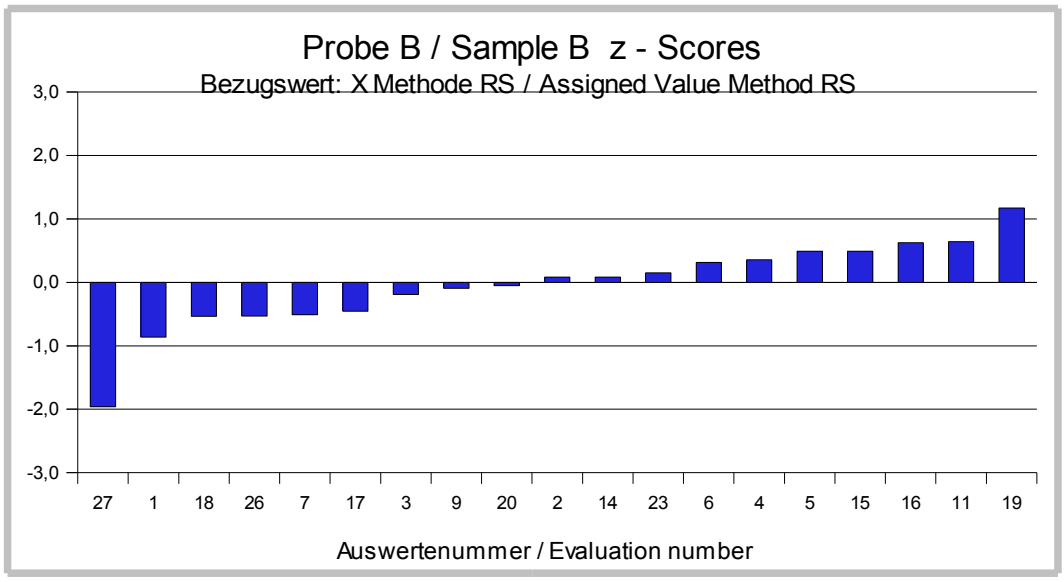

Fig. 7: z-Scores (ELISA-Results as Gluten) Assigned value robust mean of method RS (R-Biopharm, Ridascreen Fast)

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]	[%]		
16	948	38	36	180	AQ	
28	>200		39,14	196	AQ	
10	430	17	9	45	BK	
24	3000	**119**	6	30	IL	
1	>80		23,05	**115**	RS	
2	>800		30	**150**	RS	
3	> 80		28	**140**	RS	
4	1875	**74**	32	160	RS	
5	2300	**91**	33	165	RS	
6	1140	45	31,7	159	RS	
7	1541	**61**	25,65	**128**	RS	
9	1704	**67**	28,7	**144**	RS	
11	1969	**78**	34,1	171	RS	
14			30	**150**	RS	
15			33	165	RS	
16	2165	**86**	34	170	RS	
17	1833,34	**73**	26,06	**130**	RS	
18	1017,52	40	25,46	**127**	RS	
19	1600	**63**	38	190	RS	
20	1690	**67**	29	**145**	RS	
23	>400		30,5	153	RS	
26			25,5	**128**	RS	
27			15	**75**	RS	
13	530	21	25	**125**	VT	
8	>2000				div	Result converted

RA*	50-150 %	RA*	50-150 %
Number in RA	**10**	Number in RA	12
Percent in RA	**67**	Percent in RA	50

* Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen
div = not indicated / other method

Comments:
For the spiking material sample 67% of participants obtained recovery rates within the range of the AOAC-recommendation of 50-150%. For the baking mixture-sample B produced with the spiking material sample 50% of the recovery rates were in the range of acceptance.
Because a minimum gluten-content in the basic matrix "baking mixture" could not be excluded (sample A: 92% of < 5 mg/kg), it could be estimated for sample B, that the range of acceptance could be extended for the recovery rates to approximately 170%.

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		
8	negative		positive	400	2/2 (100%)	MS	
5	negative	< 0,4	positive	> 0,4	2/2 (100%)	SFA	
9	negative	<5,0	positive	975	2/2 (100%)	SFA	Outlier X_{All}, result converted
14	negative		positive		2/2 (100%)	SFA	
16	positive	<2,0	positive	54,5	1/2 (50%)	SFA	
21	negative	-	positive	102	2/2 (100%)	SFA	
2	negative		positive	190	2/2 (100%)	div	
6	negative		positive		2/2 (100%)	div	
12	negative		positive		2/2 (100%)	div	
13	negative		positive		2/2 (100%)	div	
15	negative		positive	150	2/2 (100%)	div	
19a	negative		positive		2/2 (100%)	div	
19b	negative		positive		2/2 (100%)	div	
25	negative		positive		2/2 (100%)	div	

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

Methods:
MS = AllAll, Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:
There were 93% negative results for sample A (with one positive result below LOQ) and 100% positive results for sample B by the PCR-methods for wheat. The results are in qualitative agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

Evaluation number	Wheat	z-Score X_{ALL}	Method	Remarks
	[mg/kg]	Bezug X_{ALL}		
8	400	4,9	MS	
5	> 0,4		SFA	
9	975	17,8	SFA	Outlier X_{All}, result converted
14			SFA	
16	54,5	-2,8	SFA	
21	102	-1,7	SFA	
2	190	0,2	div	
6			div	
12			div	
13			div	
15	150	-0,6	div	
19a			div	
19b			div	
25			div	

Methods:
MS = AllAll, Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Characteristics: Quantitative evaluation Wheat

Sample B

	All Results *[mg/kg]*
Assigned value	X_{ALL}
Number of results	*5 **
Robust mean (X)	179
Robust standard deviation (S^x)	151
Median	150
Target range:	
Target standard deviation ($\hat{\sigma}$)	44,8
lower limit of target range (X - 2 $\hat{\sigma}$)	89,5
upper limit of target range (X + 2 $\hat{\sigma}$)	269
Quotient $S^x/\hat{\sigma}$	*3,4*
Standard uncertainty u_X	*84,4*
Quotient $u_X/\hat{\sigma}$	*1,9*
Number of results in the target range	*3 (60%)*

* Result 9 was excluded

Comments:
There were 6 quantitative results, thereof one outlier which was excluded. The evaluation of all methods showed a high variability of results. The quotient $S^x/\hat{\sigma}$ was above 3. The comparability of results was limited.

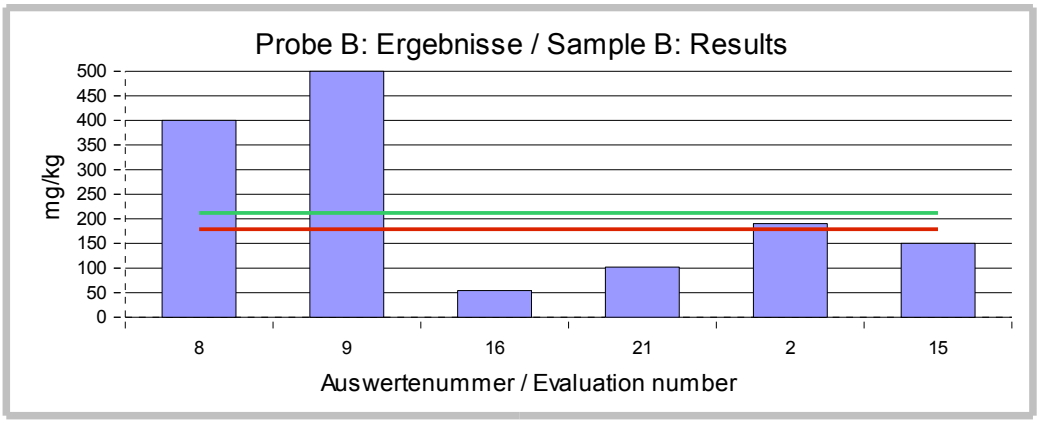

Fig. 8: PCR-Results Wheat
green line = Spiking level
blue line = Assigned value robust mean results all methods

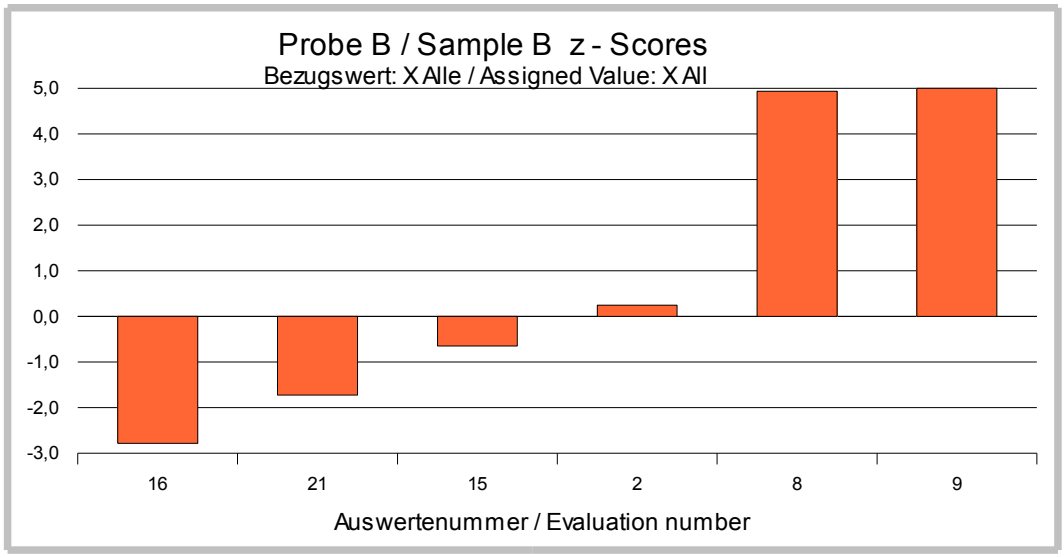

Fig. 9: z-Scores (PCR-Results as Wheat)
Assigned value robust mean of all results

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]	[%]		
8	73000	275	400	189	MS	
5	> 0,4		> 0,4		SFA	
9	342420	1292	975	460	SFA	Outlier X_{All}, result converted
14					SFA	
16	11068	42	54,5	26	SFA	
21	-		102	48	SFA	
2	167000	630	190	**90**	div	
6					div	
12					div	
13					div	
15			150	**71**	div	
19a					div	
19b					div	
25					div	

	RA*	50-150 %	RA*	50-150 %
	Number in RA	**0**	Number in RA	2
	Percent in RA	**0**	Percent in RA	33

* Range of acceptance of AOAC for allergen ELISAs

Methods:
MS = AllAll, Microsynth
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the spiking material sample none of the of participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the baking mixture-sample B produced with the spiking material sample two recovery rates were in the range acceptance.

5. Documentation

Details by the participants

5.1 ELISA: Soya

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitatives Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
3	-	<0,27	-	4,1	-	521,1	Given as	AQ	ROMERLABS
4	negative	<2,5	positive	12	positive	3025	Soyprotein	ES	ELISA-Systems Soy (ESSOYPRD-48)
5	negative	< 2,5	positive	12	positive	3400	Soyprotein	ES	ELISA-Systems Soy (ESSOYPRD-48)
6	negative	<1.25ppm	positive	9.4ppm	positive	>25ppm	Soyprotein	ES	ELISA-Systems Soy (ESSOYPRD-48)
26	negative		positive	5,85	-		Soyprotein	ES	ELISA-Systems Soy (ESSOYPRD-48)
24	negative		positive	410	positive	47000	Soyflour	IL	Immunolab Soja ELISA SOJ-E01
1	negative	<2,5	positive	216,58	positive	32200	Soy protein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
9	negative	<2,5	positive	103	positive	9.047	Given as Soy protein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
11	-	<2,5	-	194,8	-	29989	Given as	RS	r-biopharm, Lot: 14024
16	negative	<6,25	positive	514	positive	74325	Sojabohne	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
17	--	<2,5	--	168,62	--	26738,94	soya protein	RS	RIDASCREEN Fast Soya, r-biopharm R7102
18	-	< 5.00	-	138,04	-	3400	Soy protein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
20	-	< LOD	-	209	-	18166	Soy protein	RS	R7102 FAST Soya r-Biopharm AG
22	negative	<2,5	positive	104	positive	29714	Soyprotein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
28	-	<NG	-	>20ppm	-	>20 ppm	Soy protein	RS	Ridascreen Fast Soja / Soya (R7102), r-Biopharm
7	negative	<2.5	positive	19,15	positive	3400	Soyflour	VT	Veratox Soy, Neogen
10	negative		positive		positive		Given as	VT	VERATOX/NEOGEN
13	negative	<2.5	positive	26	positive	2500 (ca. 360	Soyflour	VT	Veratox Soyflour, Neogen
19	negative	<2,5	positive	28	positive	5000	Soyflour	VT	Neogen (Veratox)

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA Systems
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Other details to the Methods

Evaluation number	Meth. Abr.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Antibody	e.g. Extraction Solution / Timet / Temperature	
3	AQ			
4	ES	Anti-Soy Trypsin Inhibitor and other soy proteins	kit protocol	
5	ES			
6	ES			
26	ES			Mean value reported. Spiking mat. Not analyzed due to risk of contamination.
24	IL			
1	RS		Sample preparation and assay conduction according to test kit manual	
9	RS		Extraction according to manual	Sample C: preparation of 0,1g instead of 1,0g sample wheight: result 34.919mg/kg
11	RS		according to kit manual	Spiking 1:5000 dilution
16	RS		according to manual extraction of polyphenol containing foods (with addition of Casein and PVP) for samples A and B	Dotierprobe 1/3000 verd. gemessen
17	RS		according to kit	
18	RS		according to protocol RIDASCREEN FAST Soya 12-08-06	Mean of 6 determinations
20	RS	specific	according to manual 9.4 tannin- and polyphenol containing foods, e.g. chocolate	
22	RS	Soya protein antibody	Spiking sample: Mercaptoethanol 10 minutes at 100 °C and buffer; Samples A and B: Mercaptoethanol 10 minutes at 100 °C, buffer and casein-polyvinylpyrrolidon	Spiking Sample was diluted x 10000 and Sample B was diluted x 10
28	RS		according to kit manual	Sample B quantification: 115,2 ppm
7	VT	Soy proteins	As Kit Instructions	Sample C is heavily diluted and therfore the result is only an estimate
10	VT	SOY		
13	VT			
19	VT		according to manual	

5.2 ELISA: Gluten

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitatives Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
16	negative	<4	positive	36	positive	948	Gluten	AQ	AgraQuant Gluten G12, RomerLabs
28	-	6,8	-	39,14	-	>200ppm	Gluten	AQ	AgraQuant Gluten G12, RomerLabs
10	negative		-	9	-	430	Given as	BK	BIOKITS/NEOGEN
24	negative		positive	6	positive	3000	Gluten	IL	Immunolab Gliadin GLU-E01
1	negative	<5	positive	23,05	positive	>80	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
2	negative		positive	30	positive	>800	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
3	-	<5,0	-	28	-	> 80	Given as	RS	R-BIOPHARM
4	negative	<5,0	positive	32	positive	1875	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
5	negative	< 3	positive	33	positive	2300	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
6	negative	<3ppm	positive	31.7ppm	positive	1140ppm	Gliadin	RS	Ridascreen Gluten (R7001), r-Biopharm
7	negative	<5	positive	25,65	positive	1541	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
9	negative	<5,0	positive	28,7	positive	1.704	given as Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
11	-	<5,0	-	34,1	-	1969	Angabe als	RS	r-biopharm, Lot: 14383
14	negative		positive	30	positive		Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
15	-		positive	33	-		Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
16	positive	6,3	positive	34	positive	2165	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
17	--	<5,0	--	26,06	--	1833,34	gluten	RS	RIDASCREEN Gliadin, r-biopharm R7001
18	-	< 5.00	-	25,46	-	1017,52	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
19	negative	<5	positive	38	positive	1600	Gluten	RS	R-biopharm (R7001)
20	-	< LOD	-	29	-	1690	Gluten	RS	R7001 Gliadin r-Biopharm AG
23	negative	<5	positive	30,5	positive	>400	Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
26	negative		positive	25,5	-		Gluten	RS	Ridascreen Gluten (R7001), r-Biopharm
27	-	<3	-	15	-		Given as	RS	Ridascreen Gluten (R7001), r-Biopharm
13	negative	<5	positive	25	positive	530	Gluten	VT	Veratox Gliadin R5, Neogen
8	negative		negative		positive	>1000	Gliadin	div	in house

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
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 / Timet / Temperature	
16	AQ		according to Manual with fish gelatine for sample A and B	Spiking sample measure 1/40 diluted
28	AQ	G12	according to kit manual	Spiking sample quantification : 1809 ppm
10	BK	GLUTEN		
24	IL			
1	RS		Sample preparation and assay conduction according to test kit manual. Extraction solution R7099	Sample A with RIDA®QUICK Gliadin R7004 (qualitative)
2	RS		Coktail Solution & 80% Ethanol/2 hs/50°C&25°C	Extraction and ELISA for postive samples (B and spiking sample) repeated twice. The additional dilution (1:10) was not measurable for the spiking sample. The gluten content was too high. Content should be >800ppm.
3	RS	R5		We have problems with the matrix
4	RS	R5	kit protocol (Mendez Cocktail)	
5	RS			
6	RS	R5		
7	RS	gliadin from wheat and corresponding prolamines for rye and barley	As Kit Instructions	Sample C is heavily diluted and therfore the result is only an estimate
9	RS		Extraction with Cocktail solution according to kit manual	Sample C: preparation of 0,025g instead of 0,25g sample wheight : result 4.553mg/kg
11	RS		according to kit manual	Spiking diluted 1:50
14	RS	R5	according to kit manual	30 ± 8 mg/kg
15	RS			
16	RS		according to Manual with Cocktail solution	Spiking sample measured 1/40 diluted
17	RS		according to kit	
18	RS		according to protocol RIDASCREEN Gliadin 12-04-18, Extraction with Cocktail (patented) (R7006, official AOAC-Method)	mean of 2 determinations
19	RS		according to kit manual	
20	RS	monoclonal	according to manual Cocktail preparation	
23	RS		Extraction with Coktail solution (AOAC Method) Extraction sample B with milk powder	
26	RS			Mean value reported. Spiking mat. Not analyzed due to risk of contamination.
27	RS			
13	VT		without extraction additive	with addition of extraction additive (for presence of buckwheat, herbs etc. necessary) for spiking sample: 970 mg/kg
8	div	Rabbit polyclonal to Gliadin	Methanol 70%	

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
14	negative		positive		positive		DNA-Soya	ASU-1	andere: L 00.00-105
23	negative		positive		positive		DNA-Soya	ASU-1	ASU L 00.00-105
15	negative		positive	438	-	n.u.	Soyflour	ASU-2	Amtliche Sammlung von Untersuchungsverfahren: Methode L 08.00-59.
5	-		positive	> 5	positive	> 5	DNA-Soya	SFA	Sure Food Allergen, Congen / r-Biopharm
16	positive	2,1	positive	231	positive	55859	Soybean, total	SFA	Sure Food Allergen, Congen / r-Biopharm
21	positive	21,7	positive	> 400	-	-	Soybean, total	SFA	Sure Food Allergen, Congen / r-Biopharm
2	negative		positive	1333	positive	323500	taxonomic DNA/20ng/µl total DNA sample B= 0,133% spiking sample= 32,35%	div	Quanti>Tect MasterMix No ROX, UNG Schritt
6	negative	-	positive	+	positive	+	DNA-Soya	div	internal method
8	negative		positive	1400	positive	170000	Soyflour	div	AllGetrid
12	positive		positive		positive			div	
13	negative		positive		positive		DNA-Soya	div	
19	negative	-	positive	-	positive	-	DNA-Soya	div	internal method
25	negative		positive		positive		given as	div	in-house method

Methods:

ASU-1 = ASU L 00.00-105, ASU-2 = ASU L 08.00-59
SFA = Sure Food Allergen, 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 Solution / Timet / Temperature	
14	ASU-1	Lectin	SureFood PREP Allergen	
23	ASU-1	Lectin-Gen	Extraction with Machery& Nagel Nucleospin Food Kit 2 g sample weight	
15	ASU-2	Soja-Lectin		Spiking sample not analysed and used for quantification respectively. Matrix-calibrators: rice cake, spiked with 400 ppm wheat and soyaflour
5	SFA			
16	SFA		Sure Food Prep allergen 100mg according to manual	Spiking sample measured 1/30 diluted
21	SFA	-	Extraktion mittels SureFood® PREP Allergen	SureFood® ALLERGEN QUANT Soya (S3201)
2	div	Lectin Gen	Nucleospin Food Kit with own optimisations / columns CleanUp / RealTIme PCR Mulitplex System AllAllA (Microsynth AG, Schweiz) / 45 cycles; determination in 20ng/µl total DNA photometric regulated to 100ng DNA/PCR rxn	
6	div		internal method	
8	div	63bp	Wizard/ Rotorgene6000	
12	div		First-DNA all tissue Kit, real-time PCR	sample A positive at LOD
13	div	Lectin		
19	div		DNA Extraction: CTAB+ProtK, Chloroform, Wizard, Realtime-PCR 45 Cycles	
25	div		RNAse, Proteinase, Silica columns, Real Time PCR, 45 cycles	

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
8	negative		positive	400	positive	73000	wheatflour	MS	AllAllC
5	negative	< 0,4	positive	> 0,4	positive	> 0,4	Wheat, rye, barley, oat, spelt, kamut DNA	SFA	Sure Food Allergen, Congen / r-Biopharm
9	negative	<5,0	positive	93	positive	32.667	given as gluten	SFA	SureFood Allergen Quant Gluten (S3206), r-Biopharm
14	negative		positive		positive		other: DNA of gluten-containing cereals	SFA	Sure Food Allergen, Congen / r-Biopharm
16	positive	<2,0	positive	54,5	positive	11068	gluten containing cereals	SFA	Sure Food Allergen, Congen / r-Biopharm
21	negative	-	positive	102	-	-	gluten containing cereals	SFA	Sure Food Allergen, Congen / r-Biopharm
2	negative		positive	190	positive	167000	taxonomic DNA/20ng/µl total DNA sample B= 0,019% spiking sample= 16,7%	div	Quanti>Tect MasterMix No ROX, UNG Schritt
6	negative	-	positive	+	positive	+	DNA-Wheat	div	internal method
12	negative		positive		positive			div	
13	negative		positive		positive		DNA-Glia-Wheat	div	
15	negative		positive	150	-	n.u.	wheatflour	div	Alary et al. Cereal Chemistry 79 (4), 2002, 553-558.
19a	negative	-	positive	-	positive	-	DNA-Wheat	div	internal method
19b	negative	-	positive	-	positive	-	Wheat-, Rye-, Barley-DNA	div	internal method
25	negative		positive		positive		given as	div	in-house-method

Methods:

MS = AllAll, Microsynth
SFA = Sure Food Allergen, 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 Solution / Timet / Temperature	
8	MS	81bp, K00821	Wizard/ Rotorgene6000	
5	SFA			
9	SFA		SureFood Prep Allergen (S1012), r-Biopharm, with SureFood QUANTARD Allergen 40 (S3301) as reference	Sample A: Cp-values below Standard S4; preparation 1= 0,44mg/kg; preparation 2= 7,79mg/kg
14	SFA		SureFood PREP Allergen	
16	SFA		Sure Food Prep allergen 100mg according to Manual	Spiking sample measured 1/30 diluted
21	SFA	-	Extraction with SureFood® PREP Allergen	SureFood® ALLERGEN QUANT Gluten (S3206)
2	div		Nucleospin Food Kit with own optimisations / columns CleanUp / RealTImePCR Mulitplex System in development / 45 cycles; determination in 20ng/µl total DNA photometric regulated to 100ng DNA/PCR rxn	
6	div		internal method	
12	div			
13	div	Gliadin Wheat		
15	div	Wheat-Lipid-Transferprotein (ltp)		Spiking sample not analysed and used for quantification respectively. Matrix-calibrators: rice cake, spiked with 400 ppm wheat and soyaflour. Lowest quantification range (relatively high deviation of results
19a	div		DNA Extraction: CTAB+ProtK, Chloroform, Wizard, End point PCR 45 Cycles + Agarose gel	
19b	div		DNA Extraktion: CTAB+ProtK, Chloroform, Wizard, Endpunkt PCR 45 Cyclen + Agarose gel	
25	div		RNAse, Proteinase, Silica columns, Real Time PCR, 45 cycles	

6. Index of participant laboratories

Teilnehmer / Participants	Ort Town	Land / Country
		FRANCE
		SWITZERLAND
		SPAIN
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		ITALY
		SWEDEN
		GERMANY
		NEW ZEALAND
		GERMANY
		AUSTRIA
		GERMANY
		GERMANY
		GERMANY
		SWITZERLAND
		SWITZERLAND
		GERMANY
		AUSTRIA
		UNITED KINGDOM
		ITALY
		SPAIN
		AUSTRALIA
		GERMANY
		GERMANY
		NETHERLANDS

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

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