DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

04/2014

Allergens IV:

Celery, Mustard and Sesame

in Instant Soup Powder

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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 material is a common in commerce "broccoli cream soup" powder. The basic composition of both sample A and sample B was the same (see table 1). The spiking material sample containing celery, mustard and sesame 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**
Broccoli-Cream Soup Powder Ingredients: Vegetable fat, vegetabeles (broccoli 7%, cauliflower, onions, leek, spinach), rice flour, modified starch, whey powder, wheat flour, iodine salt, relish (soya), lactose, milk protein, thickener: guar, stabilizer: potassium phosphate, spices, emulgator: mono- and diglycerides from fatty acids, garlic, aroma, acidifier: citric acid Nutrients per 100 g powder: Protein 9,2 g, carbohydrates 40 g, fat 28 g	100 g/100 g	99,7 g/100 g

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Sample B**
Potato flour	93,6 %	0,26 %
Celery root powder	24800 mg/kg (2,48 %)	68 mg/kg
Brown Mustard flour	19700 mg/kg (1,97 %)	54 mg/kg
Sesame paste (Tahina) Ingredients: Sesame seed ground Nutrients per 100 g: Protein 26 g, carbohydrates 11 g, fat 60 g	16200 mg/kg (1,62 %)	45 mg/kg

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).

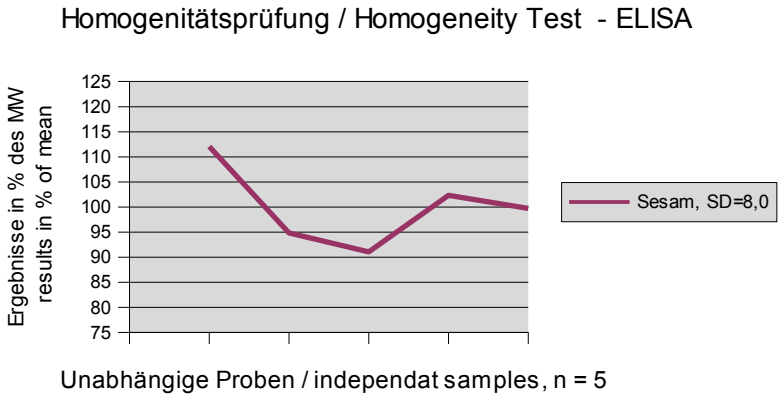

Fig. 1: Testing of homogeneity of DLA-sample B
Results are given in percent of the arithmetic mean

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 33th week of 2014. The testing method was optional. The tests should be finished at September 29th 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. mustard or sesame 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.

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

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.

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%.

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 given as mustard protein or sesame protein were converted to mustard and sesame. When possible the information supplied by the test kit manufacturer was used. A protein content of 26% for mustard and 25% for sesame was taken.

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 Celery

4.1.1 ELISA-Results: Celery

Comments:
None of the participants used the ELISA method for determination of celery.

4.1.2 PCR-Results: Celery

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		
22	negative		negative		-	ASU	
25	negative		negative		-	ASU	
26	negative		negative		-	ASU	
4	negative	< 0,08	positive	0,2	-	BD	
31	positive	0,06	positive	0,05	-	BD	
34	negative		negative		-	MS	
6	negative	<LOD	negative	<LOD	-	PL	
1	positive	/	positive	/	-	SFA	
9	positive		positive		-	SFA	
12	positive	> / <LOQ	positive	> / <LOQ	-	SFA	
16	negative		negative		-	SFA	
17	negative		negative		-	SFA	
19	positive		positive		-	SFA	
27	negative	≤ 0,4	negative	≤ 0,4	-	SFA	
28	negative	-	negative	-	-	SFA	
3	negative		negative		-	div	
7	positive		positive		-	div	
14	negative		negative		-	div	
18	negative		positive		-	div	Sample B: traces positive
20	positive	-	positive	-	-	div	
21	positive		positive		-	div	
23	negative		positive		-	div	
24	negative		negative		-	div	
29	negative		negative		-	div	

	Sample A		Sample B	
Number positive	8		11	
Number negative	16		13	
Percent positive	33		46	
Percent negative	67		54	
Consensus	none		none	

Methods:

ASU = ASU §64 Methode
BD = Biotecon Diagnostics
MS = All, Microsynth
PL = Planton GmbH
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the detection of celery by PCR methods for both samples no consensus value was obtained. Even in the spiking material sample only very low DNA content was detectable. Therefore no qualitative valuation of results was done.

For quantitative evaluation the number of results was too low.

The few given results of celery in the spiking material sample were not plausible. Only 6 out of 18 results were positive. None of the participants determined more than 0,4 mg/kg celery. The results are shown in the documentation.

The spiking material contained approximately 2,5% celery root powder. The celery powder contained 8,4% protein and a qualitatively detected amount of celery specific DNA (PCR / gel electrophoresis).
The used celery powder was purchased from a common food retailer. Details of food processing of the powder are unknown.
Manufacturing procedures such as heating and acidic pH-values may cause DNA degradation and lead to a decreased detectability of DNA.

In the German official method ASU § 64 L 08.00-56 a higher false-negative rate and a decreased detectability of celery root powder in contrast to other parts of celery was described (22). The limit of detection was 50 mg/kg for celery root powder and 10 mg/kg for celery seeds. Reasons according to the authors could be a lower content of DNA and/or less efficiency of DNA-extraction from celery root powder.

4.2 Proficiency Test Mustard

4.2.1 ELISA-Results: Mustard

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	< 2	positive	107	2/2 (100%)*	AQ	result converted
13	negative	< 2	positive	25,15	2/2 (100%)*	AQ	
23	negative		positive	14	2/2 (100%)*	AQ	
1	negative	< 0,5	positive	> 10	2/2 (100%)*	ES	
8a	negative	< 2,5	positive	42	2/2 (100%)*	ES	result converted
11	negative	< 1	positive	31	2/2 (100%)*	ES	result converted
14	negative		positive	43,5	2/2 (100%)*	ES	result converted
4	negative	< 2	positive	34,3	2/2 (100%)*	IL	
9	positive	1,3	positive	84,2	2/2 (100%)**	RS	
10	positive	1,58	positive	39,96	2/2 (100%)**	RS	
15	positive	0,89	positive	159,3	2/2 (100%)**	RS	outlier X_{All}
22	negative	< 5	positive	103,3	1/2 (50%)**	RS	
26	positive	1	positive	100	2/2 (100%)**	RS	
30	positive	1,32	positive	143,33	2/2 (100%)**	RS	
8b	negative	< 2,5	positive	79	2/2 (100%)*	VT	
12	negative	< LOQ	positive	58	2/2 (100%)*	VT	
32	negative	< 2,5	positive	72,5	2/2 (100%)*	VT	
33	negative	nd	positive	97	2/2 (100%)*	VT	

	Sample A		Sample B	
Number positive	5		18	
Number negative	13		0	
Percent positive	28		100	
Percent negative	72		0	
Consensus	neg / pos		positive	

* results without method RS = consensus „negative“ (s. comments)

** results of method RS are internally consistent = consensus „positive“ (s. comments)

Methods:

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

Comments:

There were 72% negative results for sample A and 100% positive results for sample B by the ELISA-methods.
The positive results for sample A were obtained by method RS. Within

method RS the consensus value is "positive" for sample A. For all other methods it is "negative". The results were all < 5 mg/kg.

Quantitative valuation of results: Sample A

Evaluation number	Mustard	z-Score X_{RS}	Method	Remarks
	[mg/kg]	$X_{Method\ RS}$		
9	1,3	0,3	RS	
10	1,58	1,2	RS	
15	0,89	-1,1	RS	
22	< 5		RS	not valuated
26	1	-0,7	RS	
30	1,32	0,3	RS	

Methods:
RS = Ridascreen®, R-Biopharm

Characteristics: Quantitative evaluation Mustard

Sample A

	Method RS *[mg/kg]*
Assigned value	$x_{Method\ RS}$
Number of results	*5*
Robust mean (X)	1,22
Robust standard deviation (S^x)	0,31
Median	1,30
Target range:	
Target standard deviation ($\hat{\sigma}$)	0,31
lower limit of target range (X - 2 $\hat{\sigma}$)	0,61
upper limit of target range (X + 2 $\hat{\sigma}$)	1,83
Quotient $S^x/\hat{\sigma}$	*1,0*
Standard uncertainty u_X	*0,17*
Quotient $u_X/\hat{\sigma}$	*0,57*
Number of results in the target range	*5 (100%)*

Method:
RS = R-Biopharm, Ridascreen®

Comments:
For sample A the only positive results were obtained by method RS. The evaluation showed a low variability. The quotient $S^x/\hat{\sigma}$ was clearly below 2,0. No mustard was added to sample A as an ingredient or spiking material.

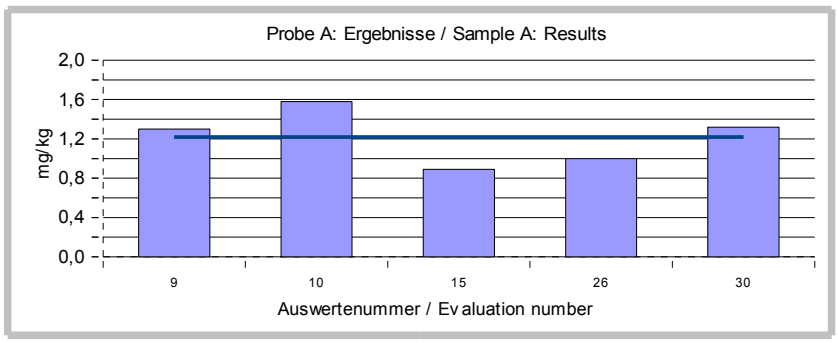

Fig. 2: ELISA-Results Mustard method RS (sample A)
blue line = Assigned value robust mean results method RS

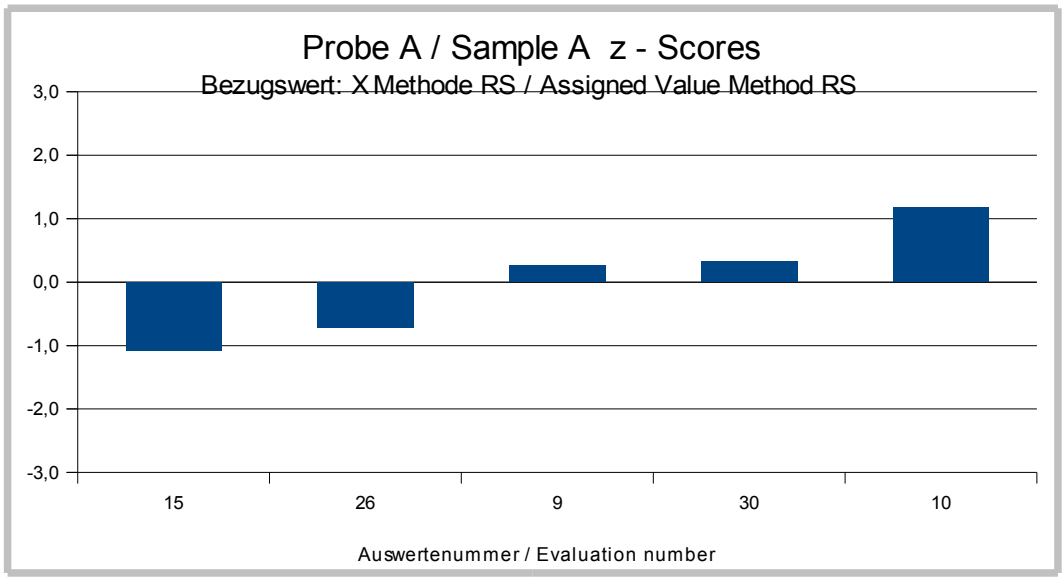

Fig. 3: z-Scores sample A (ELISA-Results as Mustard) Assigned value robust mean of method RS (Ridascreen)

Quantitative valuation of results: Sample B

Evaluation number	Mustard	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
3	107	2,0		AQ	result converted
13	25,15	-2,6		AQ	
23	14	-3,2		AQ	
1	> 10			ES	
8a	42	-1,6		ES	result converted
11	31	-2,2		ES	result converted
14	43,5	-1,5		ES	result converted
4	34,3	-2,1		IL	
9	84,2	0,8	-0,8	RS	
10	39,96	-1,7	-2,5	RS	
15	159,3	5,0	2,1	RS	outlier X_{All}
22	103,3	1,8	-0,1	RS	
26	100	1,6	-0,2	RS	
30	143,33	4,1	1,5	RS	
8b	79	0,5		VT	
12	58	-0,7		VT	
32	72,5	0,1		VT	
33	97	1,5		VT	

Methods:

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

Characteristics: Quantitative evaluation Mustard

Sample B

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*17*	*6*
Robust mean (X)	70,8	105
Robust standard deviation (S^x)	43,6	48,4
Median	72,5	102
Target range:		
Target standard deviation ($\hat{\sigma}$)	17,7	26,3
lower limit of target range (X - 2 $\hat{\sigma}$)	35,4	52,5
upper limit of target range (X + 2 $\hat{\sigma}$)	106	158
Quotient $S^x/\hat{\sigma}$	*2,5*	*1,8*
Standard uncertainty u_X	*13,2*	*24,7*
Quotient $u_X/\hat{\sigma}$	*0,75*	*0,94*
Number of results in the target range	*11 (65%)*	*4 (67%)*

Method:
RS1 = R-Biopharm, Ridascreen®

Comments:
The evaluation of all methods showed a slightly increased variability. The quotient $S^x/\hat{\sigma}$ was above 2,0. For method RS the quotient was below 2,0.
The mean of the evaluation of all results was about one third higher than the spiking level, while the results of the method RS were two times higher than the spiking level (s. also "Recovery rates of Mustard" p.24).

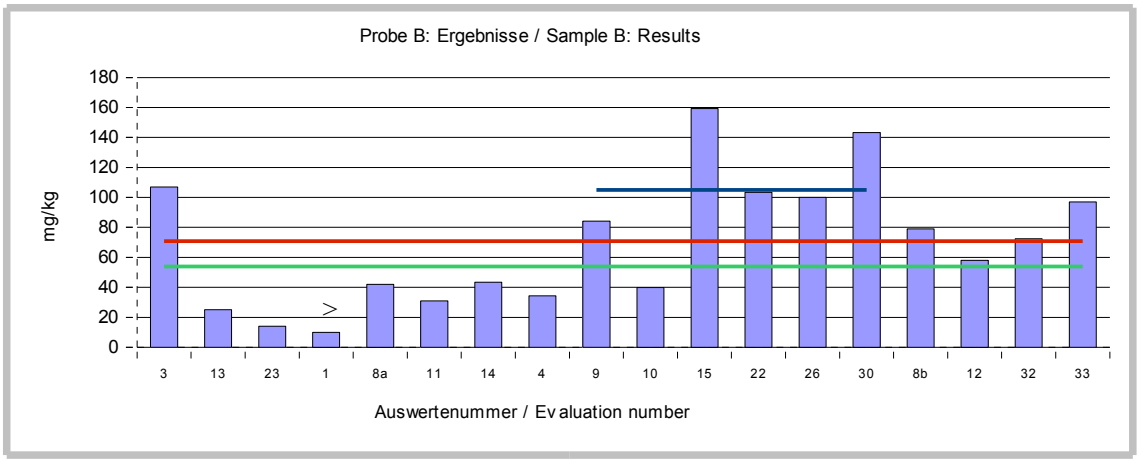

Fig. 4: ELISA-Results Mustard (sample B)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

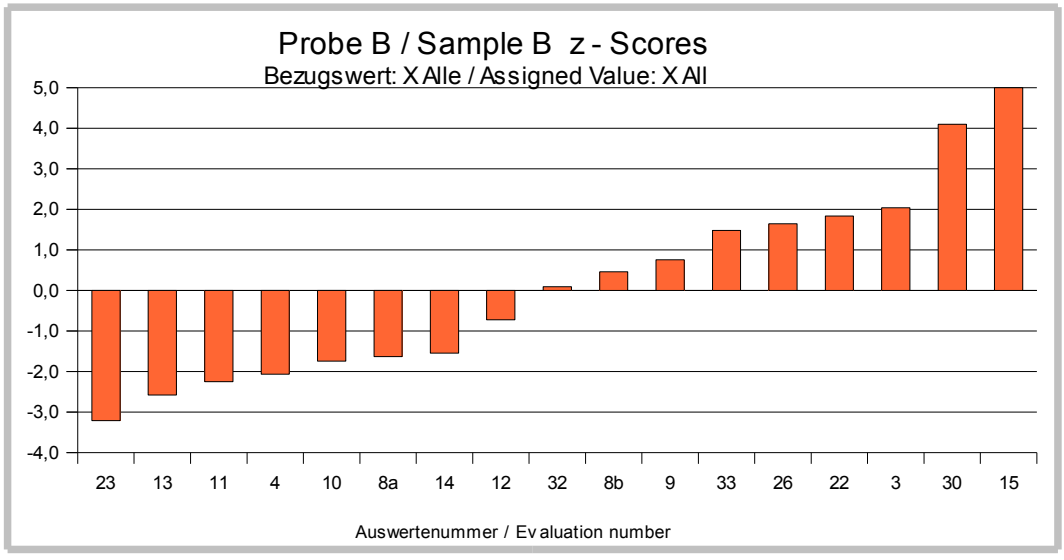

Fig. 5: z-Scores sample B (ELISA-Results as Mustard)
Assigned value robust mean of all results

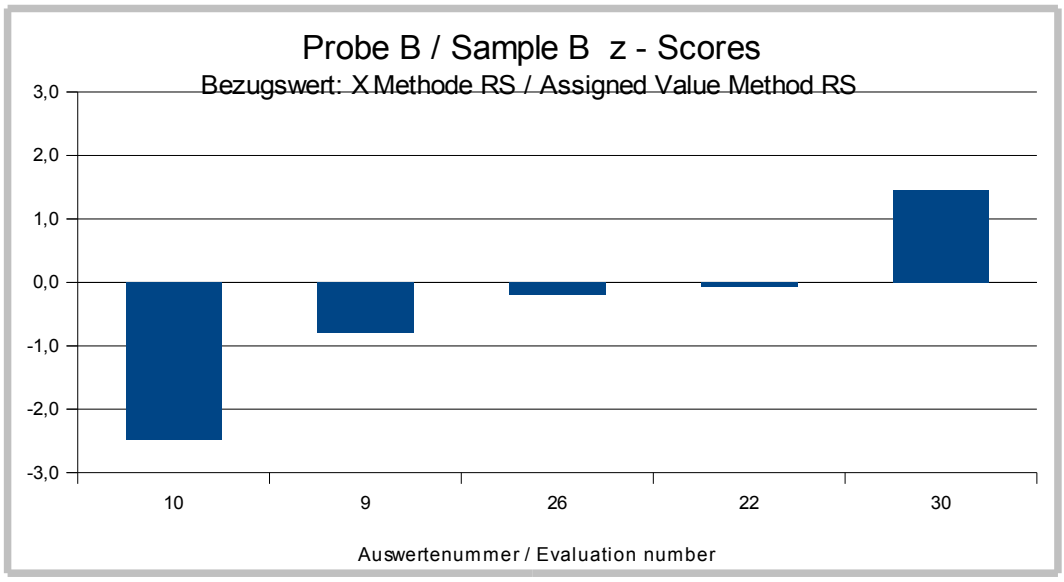

Fig. 6: z-Scores sample B (ELISA-Results as Mustard) Assigned value robust mean of method RS (R-Biopharm, Ridascreen)

Recovery Rates for Mustard:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3	56203	285	107	198	AQ	result converted
13	-		25,15	47	AQ	
23	>> BG		14	26	AQ	
1	> 10		> 10		ES	
8a	14615	**74**	42	**78**	ES	result converted
11	7692	39	31	**57**	ES	result converted
14	4538	23	43,5	**81**	ES	result converted
4	12166	**62**	34,3	**64**	IL	
9	76515	388	84,2	156	RS	
10	> Std		39,96	**74**	RS	
15	66735	339	159,3	295	RS	outlier X_{All}
22	51000	259	103,3	191	RS	
26			100	185	RS	
30	34521	175	143,33	265	RS	
12	27720	**141**	58	**107**	VT	
32	21183	**108**	72,5	**134**	VT	
33	> 25		97	180	VT	
8b	28000	**142**	79	**146**	VT	

RA*	50-150 %	RA*	50-150 %
Number in RA	**5**	Number in RA	**8**
Percent in RA	**42**	Percent in RA	**47**

Methods:

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

Comments:
For the spiking material sample 42% of participants obtained recovery rates within the range of the AOAC-recommendation of 50-150%. For the instant soup sample B produced with the spiking material sample 47% of the recovery rates were in the range of acceptance.
It should be considered that the spiking material contained brown mustard.

4.2.2 PCR-Results: Mustard

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		
21	positive		positive		2/2 (100%)	ASU	
29	negative		negative		0/2 (0%)	ASU	
6	positive	2340	positive	3385	2/2 (100%)	PL	
26	negative		negative		0/2 (0%)	QG	
1	positive	/	positive	/	2/2 (100%)	SFA	
9	positive		positive		2/2 (100%)	SFA	
11	positive	>5	positive	>5	2/2 (100%)	SFA	
17	positive		positive		2/2 (100%)	SFA	
19	positive		positive		2/2 (100%)	SFA	
27	positive	≤ 0,4	positive	≤ 0,4	2/2 (100%)	SFA	
28	positive	-	positive	-	2/2 (100%)	SFA	
32	positive		positive		2/2 (100%)	SFA	
7	positive		positive		2/2 (100%)	div	
18	positive		positive		2/2 (100%)	div	
20	positive	-	positive	-	2/2 (100%)	div	
25	positive		positive		2/2 (100%)	div	
29	positive		positive		2/2 (100%)	div	
34	positive	2000	positive	3000	2/2 (100%)	div	

	Sample A		Sample B	
Number positive	16		16	
Number negative	2		2	
Percent positive	89		89	
Percent negative	11		11	

Methods:

ASU = ASU §64 Method
PL = Planton GmbH
QG = Qiagen
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:

There were 89% positive results for sample A and sample B by the PCR-methods for mustard. The quantitative results were also in the same range for both samples. The results are therefore not in agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

There were too less numbers of results for evaluation.

Recovery Rates for Mustard:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
21					ASU	
29					ASU	
6	> LOQ		3385	6269	PL	result „given as“ not clear
26					QG	
1	/		/		SFA	
9					SFA	
11	>5		>5		SFA	
17					SFA	
19					SFA	
27	≤ 0,4		≤ 0,4		SFA	
28	-		-		SFA	
32					SFA	
7					div	
18					div	
20	-		-		div	
25					div	
29					div	
34	577000	2929	3000	5556	div	given as mustard-DNA

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

Methods:

ASU = ASU §64 Methode
PL = Planton GmbH
QG = Qiagen
SFA = Sure Food Allergen, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode

Comments:
Only two participants submitted quantitative results. With respect to mustard as a food item (seeds) the recovery rates are not plausible. It is unclear to which the quantitative results are related (DNA-copies, DNA or mustard in mg/kg).

4.3 Proficiency Test Sesame

4.3.1 ELISA-Results: Sesame

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	< 8	positive	46,4	2/2 (100%)	AQ	result converted
23	negative		positive	10	2/2 (100%)	AQ	
30	negative	< 2	positive	11,51	2/2 (100%)	AQ	
32	negative	< 2	positive	16,6	2/2 (100%)	AQ	
5	negative	< 6	positive	270	2/2 (100%)	BK	
8	negative	< 6	positive	340	2/2 (100%)	BK	
12	positive		positive		1/2 (50%)	BK	
14	negative		positive	215	2/2 (100%)	BK	
1	negative	< 2	positive	4,8	2/2 (100%)	ES	result converted
2	negative	< 2	positive	5,92	2/2 (100%)	ES	result converted
10	negative	< LOD	positive	4,52	2/2 (100%)	ES	result converted
11	negative	< 2	positive	5,6	2/2 (100%)	ES	result converted
13	negative	< 2	positive	5,40	2/2 (100%)	ES	result converted
33	negative	nd	positive	6,8	2/2 (100%)	ES	result converted
26	negative	< 0,2	positive	14	2/2 (100%)	NL	
35	negative		positive	17,96	2/2 (100%)	NL	
15	negative	< 0,24	positive	134,7	2/2 (100%)	RS	
22	negative	< 2,5	positive	235,6	2/2 (100%)	RS	
24	negative	< 4	positive	6,4	2/2 (100%)	div	result converted

	Sample A		Sample B	
Number positive	1		19	
Number negative	18		0	
Percent positive	5		100	
Percent negative	95		0	
Konsenswert	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
NL = NutriLinia, Transia
RS = Ridascreen®, R-Biopharm
div = not indicated / other method

Comments:

There were 95% negative results for sample A and 100% positive results for sample B by the ELISA-methods. Therefore the results are in qualitative agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

Evaluation number	Sesame	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
3	46,4			AQ	result converted
23	10			AQ	
30	11,51			AQ	
32	16,6			AQ	
5	270			BK	
8	340			BK	
12				BK	
14	215			BK	
1	4,8		-0,5	ES	result converted
2	5,92		0,3	ES	result converted
10	4,52		-0,7	ES	result converted
11	5,6		0,1	ES	result converted
13	5,40		-0,1	ES	result converted
33	6,8		0,9	ES	result converted
26	14			NL	
35	17,96			NL	
15	134,7			RS	
22	235,6			RS	
24	6,4			div	result converted

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
NL = NutriLinia, Transia
RS = Ridascreen®, R-Biopharm
div = not indicated / other method

Characteristics: Quantitative evaluation Sesame

Sample B

	All Results *[mg/kg]*	**Method ES** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ ES}$
Number of results	*18*	*6*
Robust mean (X)	57,8	5,51
Robust standard deviation (S^x)	87,8	0,926
Median	12,8	5,50
Target range:		
Target standard deviation ($\hat{\sigma}$)		1,38
lower limit of target range (X - 2 $\hat{\sigma}$)		2,76
upper limit of target range (X + 2 $\hat{\sigma}$)		8,27
Quotient $S^x/\hat{\sigma}$	*6,1*	*0,67*
Standard uncertainty u_X		*0,47*
Quotient $u_X/\hat{\sigma}$		*0,34*
Number of results in the target range		*6 (100%)*

Method:
ES = ELISA-Systems

Comments:
The evaluation of all methods showed a high variability with a multi-modal distribution of results. The quotient $S^x/\hat{\sigma}$ was 6,1. Therefore no statistical evaluation with respect z-scores was performed.
For the method ES the quotient $S^x/\hat{\sigma}$ was 0,67. All results were in the target range.
The mean of the evaluation the method ES was about 9 times below the spiking level (s. also "Recovery rates of Sesame" p.31).

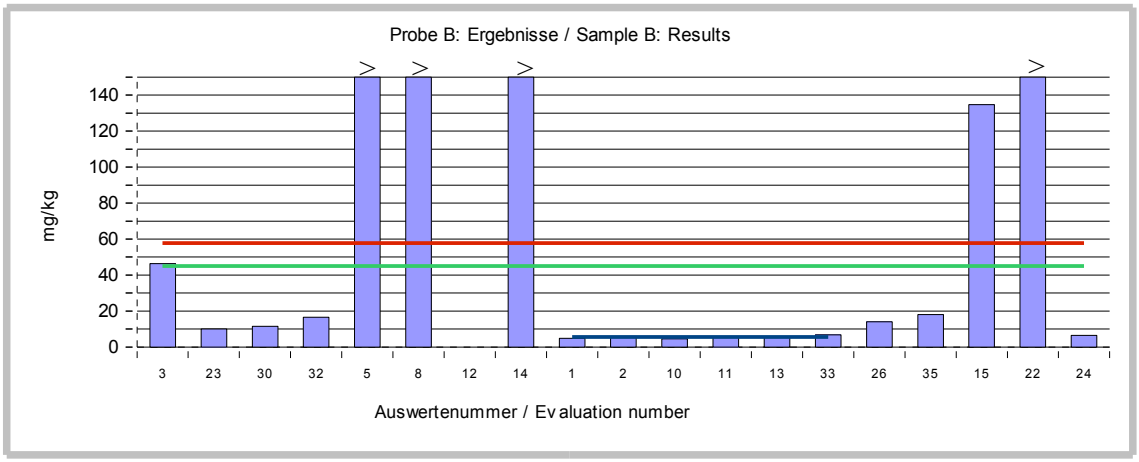

Fig. 7: ELISA-Results Sesame (sample B)
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method ES

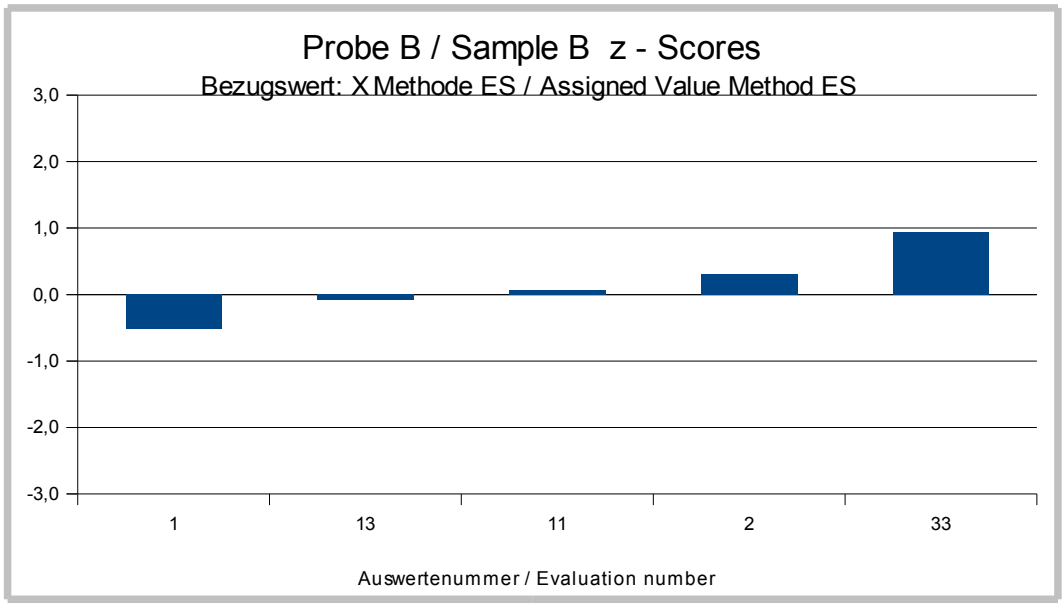

Fig. 8: z-Scores sample B (ELISA-Results as Sesame) Assigned value robust mean of method ES (ELISA-Systems)

Recovery Rates for Sesame:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3	64908	401	46,4	**103**	AQ	result converted
23	180	1	10	22	AQ	
30	15497	**96**	11,51	26	AQ	
32	13590	**84**	16,6	37	AQ	
5	62000	383	270	600	BK	
8	77000	475	340	756	BK	
12	>100000				BK	
14	31350	194	215	478	BK	
10	> Std		4,52	10	ES	result converted
1	> 20		4,8	11	ES	result converted
13	15	0,09	5,40	12	ES	result converted
11	2120	13	5,6	12	ES	result converted
2	2764	17	5,92	13	ES	result converted
33	> 20		6,8	15	ES	result converted
26			14	31	NL	
35			17,96	40	NL	
15	54960	339	134,7	299	RS	
22	59000	364	235,6	524	RS	
24			6,4	14	div	result converted

RA*	50-150 %	RA*	50-150 %
Number in RA	**2**	Number in RA	**1**
Percent in RA	**17**	Percent in RA	**6**

* Range of aceptance from AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
NL = NutriLinia, Transia
RS = Ridascreen®, R-Biopharm
div = not indicated / other method

Comments:

For the spiking material sample 2 participants obtained recovery rates within the range of the AOAC-recommendation of 50-150%. For the instant soup sample B produced with the spiking material sample one recovery rate was in the range of acceptance.

4.3.2 PCR-Results: Sesame

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		
16	negative		positive	41	2/2 (100%)	MS	
34	negative		positive	30	2/2 (100%)	MS	as Sesame-DNA
6	negative	<LOD	positive	103	2/2 (100%)	PL	
1	negative	/	positive	/	2/2 (100%)	SFA	
9	negative		positive		2/2 (100%)	SFA	
11	negative	<5	positive	>5	2/2 (100%)	SFA	
17	negative		positive		1/2 (50%)	SFA	
19	negative		positive		2/2 (100%)	SFA	
25	negative		positive		2/2 (100%)	SFA	
27	negative	≤ 0,4	positive	≤ 0,4	2/2 (100%)	SFA	
28	negative	-	positive	13,7	2/2 (100%)	SFA	
32	negative	< 1	positive	5,8	2/2 (100%)	SFA	
7	negative		positive		2/2 (100%)	div	
8	negative		positive		2/2 (100%)	div	
18	negative		positive		2/2 (100%)	div	
20	negative	-	positive	-	2/2 (100%)	div	
21	negative		positive		2/2 (100%)	div	
22	negative		positive		2/2 (100%)	div	
26	negative		positive		2/2 (100%)	div	

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

Methods:

MS = All, Microsynth
PL = Planton GmbH
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:

There were 100% negative and positive results for sample A and sample B by the PCR-methods for sesame, respectively. The results are therefore in agreement with the spiking of sample B.

Quantitative valuation of results: Sample B

There were too less numbers of results for evaluation (participant 34 submitted the result as DNA content).

Recovery Rates for Mustard:
Spiking Material Sample and Sample B

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
16	13000	**80**	41	**91**	MS	
34	39000	241	30	**67**	MS	as Sesame-DNA
6	>LOQ		103	229	PL	
1	/		/		SFA	
9					SFA	
11	>5		>5		SFA	as Sesame-DNA
17					SFA	
19					SFA	
25					SFA	
27	≤ 0,4		≤ 0,4		SFA	as Sesame-DNA
28	-		13,7	30	SFA	
32	12337	**76**	5,8	13	SFA	
7					div	
8					div	
18					div	
20	-		-		div	
21					div	
22					div	
26					div	

RA*	50-150 %	RA*	50-150 %
Number in RA	**2**	Number in RA	**2**
Percent in RA	**67**	Percent in RA	**40**

* Range of acceptance of AOAC for allergen ELISAs

Methods:

MS = All, Microsynth
PL = Planton GmbH
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:

Only five participants submitted quantitative results. Because it is unclear to which the quantitative results of participant 34 are related (DNA-copies, DNA or mustard in mg/kg) the above calculated recovery rate may not be correct (it is related to mg Sesame/kg).

5. Documentation

Details by the participants

5.1 ELISA: Celery

none

5.2 ELISA: Mustard

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
3	negative	< 2	positive	27,9	positive	14613	Protein	AQ	AgraQuant Mustard
13	negative	< 2	positive	25,15	-	-	Mustard	AQ	AgraQuant F.A.S.T. Mustard (COKAL2148F), RomerLabs
23	negative		positive	14	positive	>> BG	Mustard	AQ	AgraQuant, RomerLabs
1	negative	< 0.5ppm	positive	> 10ppm	positive	> 10ppm	Mustard	ES	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
8a	negative	< 2,5	positive	11	positive	3800	Mustard protein	ES	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
11	negative	< 1	positive	8	positive	2000	Mustard protein	ES	ELISA-Systems, Mustard Seed Protein Residue (ESMUS-48)
14	negative		-	11.3	-	1180	Mustard protein	ES	Elisa Systems Mustard seed residue MUS14-176
4	-	< 2	-	34,3	-	12166	Mustard	IL	Immunolab Senf ELISA
9	positive	1,3	positive	84,2	positive	76515	given as Mustard/Mustard powder	RS	Ridascreen Fast Senf / Mustard (R6152), r-Biopharm
10	-	1,58	-	39,96	-	> Std	Mustard	RS	Ridascreen Fast Senf / Mustard (R6152), r-Biopharm
15	positive	0,89	positive	159,3	positive	66735	Mustard	RS	Ridascreen Fast Senf / Mustard (R6152), r-Biopharm
22	negative	< 5	positive	103,3	positive	51000	Mustard powder	RS	Ridascreen Fast Senf
26	-	1	-	100	-		Mustard	RS	Ridascreen Fast Senf / Mustard (R6152), r-Biopharm
30	positive	1,32	positive	143,33	positive	34521	Mustard	RS	Ridascreen Fast Senf / Mustard (R6152), r-Biopharm
8b	negative	< 2,5	positive	79	positive	28000	Mustard	VT	Veratox Mustard Allergen, Neogen
12	negative	< LOQ	positive	58	positive	27720	Mustard	VT	Veratox Mustard Allergen, Neogen
32	negative	< 2,5	positive	72,5	positive	21183	given as	VT	Veratox Mustard Allergen, Neogen
33	negative	nd	positive	97	positive	> 25	Mustard	VT	Veratox Mustard Allergen, Neogen

Methods:

AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
RS = Ridascreen®, R-Biopharm
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			
13	AQ		-	Insufficient spiking sample to analyse for all analytes
23	AQ		According to Manual	
1	ES			
8a	ES		According to Manual	
11	ES			
14	ES	polyclonal	1g sample and 10 ml extraction buffert. 60 degrees for 15 min.	Sample B does not seem to be fully homogenous
4	IL	against mustard protein		
9	RS			
10	RS	not indicated	Spiking sample up to 1:200 diluted	Sample A: 1,63; 1,65; 1,46 Sample B: 39,90; 40,02
15	RS	mustard protein	aquaeous extraction (buffer), 10 min 60°C	
22	RS	Mustard (yellow, white, brown, black)	According to Manual	In sample A and sample B were cherry kernel size pieces, therefore the homogeneity of materials is probably not sufficient
26	RS	specific antibodies to all mustard species	According to Manual	
30	RS	Mustard (yellow, white, brown, black)	As per Kit iNstruction	Results for sample C required large dilutions and is therefore only given as an estimate
8b	VT		According to Manual	
12	VT	Mustard	According to Manual	
32	VT		According to Manual: except sample weight 1g	
33	VT		Extraction:60C pre-heated TRIS-EDTA / 15 min at 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	

5.3 ELISA: Sesame

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
3	negative	<2	positive	11,6	positive	16227	Protein	AQ	AgraQuant Sesame
23	negative		positive	10	positive	180	Sesame	AQ	AgraQuant, RomerLabs
30	negative	<2	positive	11,51	positive	15497	Sesame	AQ	AgraQuant Sesame (COKAL1948), RomerLabs
32	negative	< 2	positive	16,6	positive	13590	Sesame	AQ	AgraQuant Sesame (COKAL1948), RomerLabs
5	negative	<6	positive	270	positive	62000	Given as	BK	BioKits, Sesame Assay Kit (902070X), Neogen
8	negative	<6	positive	340	positive	77000	Sesame	BK	BioKits, Sesame Assay Kit (902070X), Neogen
12	positive		positive		positive	>100000	Sesame	BK	BioKits, Sesame Assay Kit (902070X), Neogen
14	negative		-	215	-	31350	Sesame	BK	Neogen biokits Sesame Assay kit 205974
1	negative	<0.5ppm	positive	1.2ppm	positive	>5ppm	Sesameprotein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
2	negative	<0.5	positive	1,48	positive	691	sesame seed protein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
10	-	<LOD	-	1,13	-	> Std	Sesameprotein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
11	negative	<0,5	positive	1,4	positive	530	Sesameprotein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
13	negative	< 0.5	positive	1,35	positive	3,8	Sesameprotein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
33	negative	nd	positive	1,7	positive	>5.0	Sesame seed protein	ES	ELISA-Systems, Sesame Seed Protein Residue (ESSESRD-48)
26	-	< 0,2	-	14	-		Sesame	NL	nutriLinia Sesam-E (NC-6005), Transia
35	negative		positive	17,96	positive		Sesame	NL	Transia Sesam E
15	negative	< 0,24	positive	134,7	positive	54960	Sesame	RS	RIDASCREEN®FAST Sesam
22	negative	<2,5	positive	235,6	positive	59000	Sesame	RS	Ridascreen Fast Sesam
24	negative	<1,0	-	1,6	-		Sesameprotein	div	Selection Sesame-Kits:

Methods:

AQ = AgraQuant, RomerLabs
BK = BioKits, Neogen
ES = ELISA-Systems
NL = NutriLinia, Transia
RS = Ridascreen®, R-Biopharm
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	
3	AQ			
23	AQ		According to Manual	>LOQ
30	AQ	Sesame Protein	As per Kit iNstruction	Results for sample C required large dilutions and is therefore only given as an estimate
32	AQ		According to Manual	
5	BK			
8	BK		According to Manual	
12	BK	Sesame	According to Manual	
14	BK	polyclonal	High salt Tris extraction buffer, 1g sample + 5 ml preheated buffer, 2 min RT.	
1	ES			
2	ES		in the spiking sample, dilution of 1:1000 gave the result of 0.691mg/kg result at this dilution, thus the final result is 691mg/kg. Larger dilutions- all gave result of <0.5mg/kg	
10	ES	specific anti- sesame seed 2S-albumin ab	Spiking sample was diluted up to 1:100	Sample B: 1,14; 1,12; 1,14
11	ES			
13	ES		-	-
33	ES	anti-sesame seed 2S albumin anitbodies	Extraction: PBS / 15 min @ 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	
26	NL	against Sesame proteins directed Ab	According to Manual	
35	NL	poly		
15	RS	Sesameprotein	aquaeous extraction (buffer), 10 min 60°C	
22	RS	Sesameprotein	According to Manual	see above
24	div			

5.4 PCR: Celery

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
22	negative		negative		negative		Celery-DNA	ASU	ASU §64 L 08.00-56
25	negative		negative		negative		Celery-DNA	ASU	ASU §64 L 08.00-56
26	negative		negative		-		Celery	ASU	5xQuantiFast® Pathogen PCR Fa.Qiagen Primer/Sonde:eurofins/mwg/operon. Amtliche Sammlung nach § 64 LFGB
4	negative	<0,08	positive	0,2	positive	0,1	Celery	BD	Biotecon Diagnostics GmbH
31	positive	0,06	positive	0,05	positive	0,11	Celery	BD	foodproof Celery Detection Kit
34	negative		negative		negative		Celery-DNA	MS	Köppel et al. 2012 (AllAll C, D)
6	negative	<LOD	negative	<LOD	negative	<LOD	given as	PL	PLANTON GmbH; pmApiumMat3
1	positive	/	positive	/	positive	/	Celery-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
9	positive		positive		positive		given as Celery	SFA	SureFood Allergen Celery (S3105), r-Biopharm
12	positive	>LOD, <LOQ	positive	>LOD, <LOQ	-		Celery-DNA	SFA	Congen Surefood Allergen Quant Celery
16	negative		negative		negative		Celery	SFA	Surefood Allergen QUANT Celery, Congen (via r-biopharm)
17	negative		negative		negative		given as	SFA	Sure Food Allergen Celery, Congen
19	positive		positive		-		given as	SFA	r-biopharm
27	negative	≤ 0,4	negative	≤ 0,4	negative	≤ 0,4	celery DNA	SFA	SureFood ALLERGEN Celery, CONGEN S3105
28	negative	-	negative	-	-	-	given as	SFA	S3401 SureFood® ALLERGEN 4plex Soya/Celery/Mustard+ IACS3401 SureFood® ALLERGEN 4plex Soya/Celery/Mustard+ IAC
3	negative		negative		negative		DNA	div	In-house Method
7	positive		positive		positive		given as	div	andere: Hausverfahren QB-RT-102
14	negative		negative		negative		Celery-DNA	div	In house
18	negative		Spuren positive		negative		Celery-DNA	div	Primer/Sonden TIB Molbiol
20	positive	-	positive	-	negative	-	Celery	div	other: CEN/TS 15634-2:2012
21	positive		positive		positive		Celery-DNA	div	inhouse method
23	negative		positive		-		Celery	div	Köppel et al. 2012, Eugster 2010
24	negative		negative		-		Celery-DNA	div	
29	negative		negative		negative		given as	div	inhouse method

Methods:

ASU = ASU §64 Methode
BD = Biotecon Diagnostics
MS = All, Microsynth
PL = Planton GmbH
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	
22	ASU	Mannitoldehydrogenase (101 bp)	Extraction: CTAB based extraction method followed by clean up with Wizard-Kit from Promega; Real-time PCR: 45 cycles	
25	ASU	Mannitol-Dehydrogenase	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	
26	ASU	Protein Mannitoldehydrogenase	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 cycles	
4	BD		Real Time PCR, foodproof DNA Extraktion	
31	BD		column based / foodproof Sample Preparation Kit III / 200mg sample wheight/ Real-time PCR (LC 480-II)	Quanification by Allergen RM 800 (Biotecon Diagnostics); no inhibition of internal control
34	MS	Mannitol Dehydrogenase Gene	Wizard Extraktion / Rotorgene / 45 cycles	In samples A & B 7000 mg/kg bovine-DNA measured, spiking sample negative
6	PL	s.SOP	PLANTON GmbH; CTAB; Magnetic Beads	
1	SFA		Extraction with internal method / real time PCR	
9	SFA		SureFood Prep Allergen (S1012), r-Biopharm	
12	SFA	Celery	Real time PCR	
16	SFA	Celery-DNA	according to kit manual, 45 cycles	2 Analysis
17	SFA		Real Time, 35 Cycles	NWG 0,4 ppm, DNA-Extraction Sure Food Prep PlantX
19	SFA		Real Time PCR	
27	SFA		foodproof Magnetic Preparation Kit III, BIOTECON S40013L	
28	SFA	-	LOD 1 mg/kg; DNA-Extraction with S1012 SureFood® PREP Allergen	-
3	div			
7	div		Phenol-Chloroform-Extraction	
14	div	The mannitol-dehydrogenas-gene	Extraction with Dneasy Blood&Tissue kit, Qiagen. Real time PCR with probe, 45 cylces	
18	div	Celery-Manitoldehydrogenase Gens	Real Time PCR	Ct-value 38,5
20	div	Mannitol Dehydrogenase AF67082	Extraction: Biotecon GMO sample preparation kit+alfa-amylase, +RNAase, Real-time PCR: Taqman, FAM-TAMRA, 45 cycles, Reference gene: actin	
21	div		DNA Extraction with MN Food Kit, RealTimePCR 45 Cycles	
23	div	Mannitoldehydrogenase Gene	according SLMB resp. Lit.	
24	div			
29	div			

5.5 PCR: Mustard

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
21	positive		positive		positive		Mustard -DNA	ASU	ASU §64 L 08.00-59
29	negative		negative		negative		given as	ASU	ASU §64 L 08.00-56
6	positive	2340	positive	3385	positive	> LOQ	given as	PL	PLANTON GmbH; pmBBSenf-Cy5; pmSinAlba-Hex
26	negative		negative		-		yellow Mustard	QG	5xQuantiFast® Pathogen PCR Fa.Qiagen Primer/Sonde:eurofins/mwg/operon. Official method ASU § 64 LFGB
1	positive	/	positive	/	positive	/	Mustard-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
9	positive		positive		positive		given as Mustard	SFA	SureFood Allergen Mustard (S3109), r-Biopharm
11	positive	>5	positive	>5	positive	>5	Mustard-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
17	positive		positive		positive		given as	SFA	Sure Food Allergen Mustard, Congen
19	positive		positive		-		given as	SFA	r-biopharm
27	positive	≤ 0,4	positive	≤ 0,4	positive	≤ 0,4	mustard DNA	SFA	SureFood ALLERGEN Mustard, CONGEN S3109
28	positive	-	positive	-	-	-	given as	SFA	S3401 SureFood® ALLERGEN 4plex Soya/Celery/Mustard+ IACS3401 SureFood® ALLERGEN 4plex Soya/Celery/Mustard+ IAC
32	positive		positive		positive		Mustard	SFA	Sure Food Allergen Mustard
7	positive		positive		positive		given as	div	other: in-house method QB-RT-104
18	positive		positive		positive		Mustard -DNA	div	Primer/Sonden TIB Molbiol
20	positive	-	positive	-	positive	-	Mustard	div	other: Palle-Reisch, M., et al. Food chem.138 (2013)
25	positive		positive		positive		Mustard -DNA	div	Mustorp et al. 2008 Eur Food Res Technol. 226: 771-778
29	positive		positive		positive		given as	div	Hausverfahren
34	positive	2000	positive	3000	positive	577000	Mustard -DNA	div	Palle-Reisch et al 2013

Methods:

ASU = ASU §64 Methode
PL = Planton GmbH
QG = Qiagen
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	
21	ASU		DNA Extraction with MN Food Kit, RealTimePCR 45 Cycles	
29	ASU			
6	PL	s.SOP	PLANTON GmbH; CTAB; Magnetic Beads	
26	QG	MADS-D-Protein from Sinapis alba	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 cycles	
1	SFA		Extraction with internal method / real time PCR	
9	SFA		SureFood Prep Allergen (S1012), r-Biopharm	
11	SFA			
17	SFA		Real Time, 35 Cycles	LOD 0,4 ppm, DNA-Extraction Sure Food Prep PlantX
19	SFA		Real Time PCR	
27	SFA		foodproof Magnetic Preparation Kit III, BIOTECON S40013L	
28	SFA	-	LOD 1 mg/kg; DNA-Extraction by S1012 SureFood® PREP Allergen	-
32	SFA		Sure Food Prep Allergen, qPCR, 45 cycles	
7	div		Phenol-Chloroform-Extraction	
18	div	Rubisco-Gene	Real Time PCR	
20	div	AJ415649	Extraction: Biotecon GMO sample preparation kit+alfa-amylase, +RNAase, Real-time PCR: Taqman, FAM-TAMRA, 45 cycles, Reference gene: actin	
25	div	major allergen sin a1	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	
29	div			
34	div	gypsy-like retroelement 13G42-26	Wizard Extraktion / Rotorgene / 45 Zyklen	

5.5 PCR: Sesame

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
16	negative		positive	41	positive	13000	Sesame	MS	AllAllB, KLZH / microsynth
34	negative		positive	30	positive	39000	Sesame-DNA	MS	Köppel et al. 2012 (AllAll C, D)
6	negative	<LOD	positive	103	positive	>LOQ	given as	PL	PLANTON GmbH; 5-SES-Cy5
1	negative	/	positive	/	positive	/	Sesame-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
9	negative		positive		positive		given as Sesame	SFA	SureFood Allergen Sesam (S3108), r-Biopharm
11	negative	<5	positive	>5	positive	>5	Sesame-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
17	negative		positive		positive		given as	SFA	Sure Food Allergen Sesam, Congen
19	negative		positive				given as	SFA	r-biopharm
25	negative		positive		positive		Sesame-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
27	negative	≤ 0,4	positive	≤ 0,4	positive	≤ 0,4	sesame DNA	SFA	SureFood ALLERGEN Sesame, CONGEN S3108
28	negative	-	positive	13,7	-	-	Sesame	SFA	S3208 SureFood® ALLERGEN QUANT SesameS3208 SureFood® ALLERGEN QUANT Sesame
32	negative	< 1	positive	5,8	positive	12337	Sesame	SFA	Sure Food Allergen Quant Sesam
7	negative		positive		positive		given as	div	other: in-house method QB-RT-100
8	negative		positive		positive		Sesame-DNA	div	Brzezinski, J. L., 2007. Detection of Sesame Seed DNA in Foods Using Real-Time PCR., Journal of Food Protection Vol. 70, No. 4, 1033-1036
18	negative		positive		positive		Sesame-DNA	div	Primer/Sonden TIB Molbiol
20	negative	-	positive	-	positive	-	Sesame	div	other: Köppel. R., et al., Eur Food Res Technol. 230 (2010)
21	negative		positive		positive		Sesame-DNA	div	inhouse Methode
22	negative		positive		positive		Sesame-DNA	div	Mustorp et al., 2008 Eur Food Res Technol
26	negative		positive				Sesame	div	5xQuantiFast® Pathogen PCR Fa.Qiagen Primer/Sonde: eurofins/mwg/ operon. Methode nach Mustorp et al 2007

Methods:

MS = All, Microsynth
PL = Planton GmbH
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	
16	MS	Sesame-DNA	Surefood-DNA Extraction kit, Quantifast Mastermix QIAGEN, 45 cycles	2 Analysis, Sample B 29.5ppm / 52.5 ppm, DotMaterial 11'100ppm, 14'900ppm
34	MS	Oleosin Gen	Wizard Extraction / Rotorgene / 45 cycles	
6	PL	s.SOP	PLANTON GmbH; CTAB; Magnetic Beads	
1	SFA		Extraction with internal method / real time PCR	
9	SFA		SureFood Prep Allergen (S1012), r-Biopharm	
11	SFA			
17	SFA		Real Time, 35 Cycles	LOD 0,4 ppm, DNA-Extraction Sure Food Prep PlantX
19	SFA		Real Time PCR	
25	SFA	unknown	CTAB Precipitation, QIAgen PCR Purification Kit, Real Time PCR	
27	SFA		foodproof Magnetic Preparation Kit III, BIOTECON S40013L	
28	SFA	-	LOQ 1 mg/kg; DNA-Extraction by S1012 SureFood® PREP Allergen	-
32	SFA		Sure Food Prep Allergen, qPCR, 45 cycles	
7	div		Phenol-Chloroform-Extraction	
8	div	2S Albumin Genes	CTAB-Method with further clean up (s. §64 LFBG L08.00-56)	
18	div	2S albumin Gene	Real Time PCR	
20	div	U97700	Extraction: Biotecon GMO sample preparation kit+alfa-amylase, +RNAase, Real-time PCR: Taqman, FAM-TAMRA, 45 cycles, Reference gene: actin	
21	div		DNA Extraction with MN Food Kit, RealTimePCR 45 Cycles	
22	div	2S albumin Gene (64 bp)	Extraction: CTAB based Extraction method followed by clean up with Wizard-Kit Promega; Real-time PCR: 45 cycles	
26	div	2 S Albumin	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 cycles	

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SWITZERLAND
		GERMANY
		GERMANY
		FRANCE
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		CYPRUS
		GERMANY
		GERMANY
		GERMANY
		ITALY
		SWEDEN
		GERMANY
		UK
		FINLAND
		GERMANY
		GERMANY
		ISRAEL
		SWITZERLAND
		SWITZERLAND
		GERMANY
		SWEDEN
		GERMANY
		UK
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		NETHERLANDS
		CANADA

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

7. Index of references

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs;W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
12. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
13. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003)
14. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006)
15. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007)
16. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
17. Ministry of Health and Welfare, JSM, Japan 2006
18. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen
19. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren
20. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
21. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
22. ASU § 64 L 08.00-56 Nachweis einer spezifischen DNA-Sequenz aus Sellerie (Apium graveolens) in Brühwürsten mittels Real-time-PCR (2014)
23. ASU § 64 L 18.00-22 Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014)
24. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)