DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

05/2014

Allergens V:

Peanut and Pistachio

in Pastry

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1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the 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 pastry made from a common in commerce baking mixture with additional ingredients. The basic composition of both sample A and sample B was the same (see table 1). The spiking material sample containing peanut and pistachio was added to sample A before baking. After preparation of the dough samples were baked for 60 min at approximately 170°C in an automatic baking machine.
After cooling to room temperature the samples were crushed, homogenised and portioned to approximately 25 g.

The composition of the spiking material sample and the amounts of allergens in sample B is given in table 2.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Baking mixture "Pound Cake" Ingredients: Wheat flour, sugar, wheat starch, baking agent: disodium phosphate and sodium hydrogencarbonate, rice starch, emulsifier: E475, E471 and E433, thickener: E466, aroma	53 g/100g	53 g/100g
Margarine	20 g/100g	20 g/100g
Milk	16 g/100g	16 g/100g
Eggs	12 g/100g	12 g/100g
Spiking material sample	0,58 g/100g	-

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Sample A**
Potato flour	81 %	0,47 %
Peanut mush Ingredients: Peanuts (99,2%), sea salt Nutrients per 100g: Protein 30 g	25900 mg/kg (2,59 %)	124 mg/kg
- as Peanut - thereof Peanutprotein	25700 mg/kg 7710 mg/kg	123 mg/kg 37 mg/kg
Pistachio spread Ingredients: Sugar, pistachio (30%), vegetable oils, skimmed milk powder (6%), whey powder (3%), emulsifier: lecithine, salt, aroma, colours: curcumin, copper complexes of chlorophyll	79500 mg/kg (7,95 %)	377 mg/kg
- as Pistachio - therof Pistachioprotein	23800 mg/kg 4760 mg/kg	113 mg/kg 23 mg/kg
Almond mush, white	2,04 %	0,097 %
Hazelnut spread	6,23 %	0,030 %

* related to sample weight after baking

2.1.1 Homogeneity

Homogeneity of the spiked sample A was checked by 5fold ELISA-test. The resulting standard deviation between the samples of < 15% ensured sufficient homogeneity (17, 18, 20).

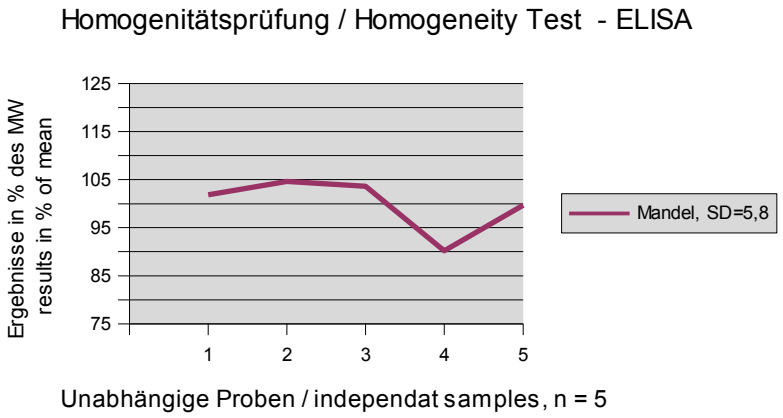

Fig. 1: Testing of homogeneity of DLA-sample A
Results are given in percent of the arithmetic mean
(tested for almond content)

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 39th week of 2014. The testing method was optional. The tests should be finished at November 7th 2014 the latest.

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. peanut or pistachio 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.
Two participants submitted no results, all other 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%.

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 peanut protein or pistachio protein were converted to almond and walnut. When possible the information supplied by the test kit manufacturer was used. A protein content of 25% for peanuts and 20% for pistachio 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 Peanut

4.1.1 ELISA-Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with Consensus Value		
12	positive	200	negative	< 4	2/2 (100%)	AQ	Result converted and mean calculated by DLA
4	positive	>30	negative	< 1	2/2 (100%)	BC	
3	positive	39	negative	< 1	2/2 (100%)	BK	
19	positive	40	negative	< 0,1	2/2 (100%)	BK	
13	positive		negative		2/2 (100%)	ES	
18	positive	75	negative		2/2 (100%)	IL	
24	positive	251	negative	< 1	2/2 (100%)	IL	outlier X_{All}
1	positive	120	negative	< 1,5	2/2 (100%)	RS	
2	positive	87,52	negative	< 2.5	2/2 (100%)	RS	
8	positive	143	negative	≤ 2,5	2/2 (100%)	RS	
9	positive	111,6	negative		2/2 (100%)	RS	
10	positive	111,5	negative	< LOD	2/2 (100%)	RS	mean calculated by DLA
11	positive	95,5	negative	< 1,5	2/2 (100%)	RS	
17	positive	106	negative	< 2,5	2/2 (100%)	RS	
20	positive	99,5	negative	< 2,5	2/2 (100%)	RS	mean calculated by DLA
15	positive	101	negative	< 2.5	2/2 (100%)	VT	

	Sample A		Sample B	
Number positive	16		0	
Number negative	0		16	
Percent positive	100		0	
Percent negative	0		100	
Consensus	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck
BK = Biokits, Neogen
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 A.

Quantitative evaluation of results: Sample A

Evaluation number	Peanut	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
12	200	3,5		AQ	Result converted and mean calculated by DLA
4	>30			BC	
3	39	-2,5		BK	
19	40	-2,5		BK	
13				ES	
18	75	-1,2		IL	
24	251	5,5		IL	outlier X_{All}
1	120	0,5	0,4	RS	
2	87,52	-0,7	-0,8	RS	
8	143	1,4	1,3	RS	
9	111,6	0,2	0,1	RS	
10	111,5	0,2	0,1	RS	mean calculated by DLA
11	95,5	-0,4	-0,5	RS	
17	106	0,0	-0,1	RS	
20	99,5	-0,2	-0,3	RS	mean calculated by DLA
15	101	-0,2		VT	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck
BK = Biokits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Characteristics: Quantitative evaluation Peanut

Sample A

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*14*	*8*
Robust mean (X)	106	108
Robust standard deviation (S^x)	46,0	16,0
Median	104	109
Target range:		
Target standard deviation ($\hat{\sigma}$)	26,5	27,0
lower limit of target range (X - 2 $\hat{\sigma}$)	53,0	54,0
upper limit of target range (X + 2 $\hat{\sigma}$)	159	162
Quotient $S^x/\hat{\sigma}$	*1,7*	*0,59*
Standard uncertainty u_X	*15*	*7,1*
Quotient $u_X/\hat{\sigma}$	*0,58*	*0,26*
Number of results in the target range	*10 (71%)*	*8 (100%)*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments:
The evaluation of all methods and the evaluation of results from method RS showed a normal or low variability, respectively. The quotients $S^x/\hat{\sigma}$ were below 2,0. The comparability of results is given.
The mean of the evaluation was about 85% and 89% of the spiking level (s. "Recovery rates of Peanut" p.18).

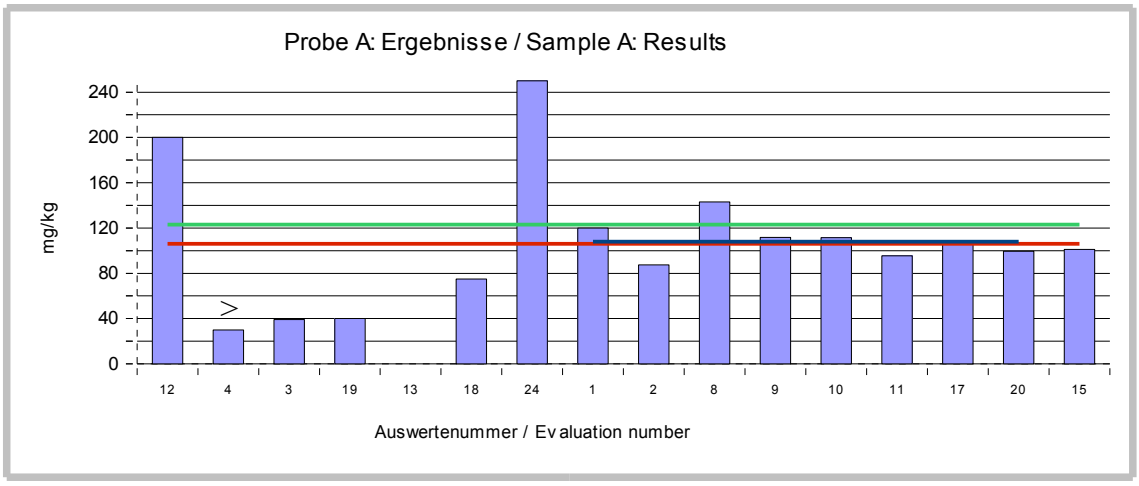

Fig. 2: ELISA-Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

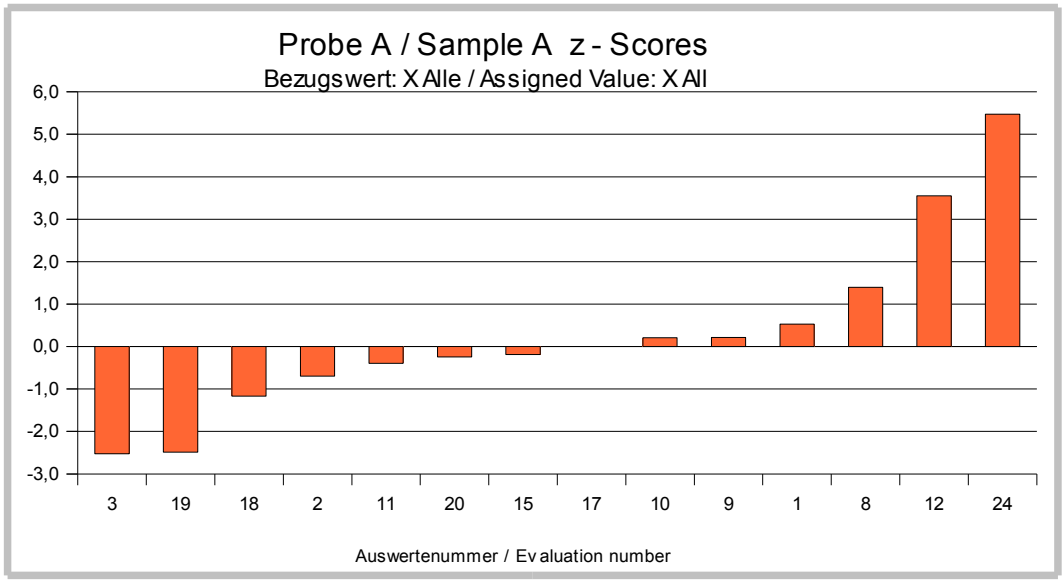

Fig. 3: z-Scores (ELISA-Results as Peanut) Assigned value robust mean of all results

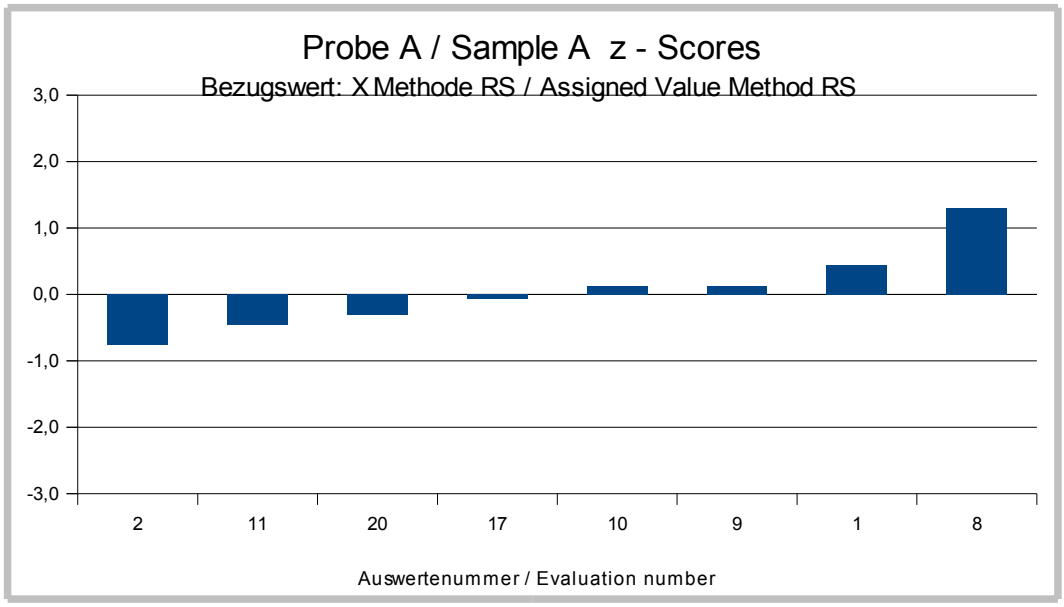

Fig. 4: z-Scores (ELISA-Results as Peanut) Assigned value robust mean of method RS (R-Biopharm, Ridascreen Fast)

Recovery Rates for Peanut:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
12	125700	489	200	163	AQ	Result converted and mean calculated by DLA
4			> 30		BC	
3	7000	27	39	32	BK	
19			40	33	BK	
13					ES	
18	98	0	75	**61**	IL	
24	139000	541	251	204	IL	outlier X_{All}
1	49000	191	120	**98**	RS	
2	50407	196	87,52	**71**	RS	
8	95010	370	143	**116**	RS	
9	52809	205	111,6	**91**	RS	
10	> 5000		111,5	**91**	RS	mean calculated by DLA
11	> 4000		95,5	**78**	RS	
17	37685	**147**	106	**86**	RS	
20	39205	153	99,5	**81**	RS	mean calculated by DLA
15	> 500		101	**82**	VT	

RA*	50-150 %	RA*	50-150 %
Number in RA	**1**	Number in RA	**10**
Percent in RA	**10**	Percent in RA	**71**

* Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck
BK = Biokits, Neogen
IL = Immunolab
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Comments:
For the spiking material sample only 1 participant obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the baked sample A produced with the spiking material sample 10 (=71%) recovery rates were in the range of acceptance.

4.1.2 PCR-Results: Peanut

Evaluation number	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample A	Method	Remarks
	pos / neg	mg/kg	pos / neg	mg/kg	pos / neg		
7	positive		negative		2/2 (100%)	ASU	
9	positive		negative		2/2 (100%)	ASU	
14	positive		negative		2/2 (100%)	ASU	
18	positive		negative		2/2 (100%)	ASU	
19	positive		negative		2/2 (100%)	ASU	
23	positive	728	negative		2/2 (100%)	MS	
5	positive	389	negative		2/2 (100%)	PL	
6	negative		positive		0/2 (0%)	QG	
1	positive	> 10	negative	< 10	2/2 (100%)	SFA	
11	positive		negative		2/2 (100%)	SFA	
12	positive	51,88	negative	< 1	2/2 (100%)	SFA	mean calculated by DLA
13	positive		negative		2/2 (100%)	SFA	
21	positive		negative		2/2 (100%)	SFA	
25	positive	-	negative	-	2/2 (100%)	SFA	
25	positive	68,8	negative	-	2/2 (100%)	SFA*	
7	positive		negative		2/2 (100%)	div	
22	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	16		1	
Number negative	1		16	
Percent positive	94		6	
Percent negative	6		94	
Consensus	positive		negative	

Methods:

ASU = ASU L 44.00-11
MS = AllAll C u. D, Microsynth
PL = Planton
QG = Qiagen
SFA = Sure Food Allergen S3402, R-Biopharm / Congen
SFA* = Sure Food Allergen S3202, S3203, R-Biopharm / Congen
div = not indicated / other method

Comments:

For the detection of peanut by PCR 94% positive results for sample A and 94% negative results for sample B were obtained. Most likely results of participant number 6 were mixed up.

Recovery Rates for Peanut:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
7					ASU	
9					ASU	
14					ASU	
18					ASU	
19					ASU	
23	269468	1049	728	592	MS	
5	> LOQ		389	316	PL	
6					QG	
1	> 10		> 10		SFA	
11					SFA	
12	27141,58	**106**	51,88	42	SFA	mean calculated by DLA
13					SFA	
21					SFA	
25	-		-		SFA	
25	-		68,8	**56**	SFA*	
7					div	
22					div	

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

* Range of acceptance of AOAC for allergen ELISAs

Methods:
ASU = ASU L 44.00-11
MS = AllAll C u. D, Microsynth
PL = Planton
QG = Qiagen
SFA = Sure Food Allergen S3402, R-Biopharm / Congen
SFA* = Sure Food Allergen S3202, S3203, 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 baked sample B spiked with the spiking material sample one of the recovery rates was in the range of acceptance too.

4.2 Proficiency Test Pistachio

4.2.1 ELISA-Results: Pistachio

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		
2	positive	6,11	negative	< 1	2/2 (100%)	AQ	
10	positive	16,6	negative	< LOD	2/2 (100%)	AQ	Mean calculated by DLA
24	positive	14	negative	< 1	2/2 (100%)	IL	
9	positive	10,7	negative		2/2 (100%)	NL	

	Probe A		Probe B	
Number positive	4		0	
Number negative	0		4	
Percent positive	100		0	
Percent negative	0		100	
Consensus	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = NutriLinia, Transia

Comments:

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

Quantitative valuation of results: Sample A

Because of the low number of the quantitative results an statistical evaluation was not done.

Recovery Rates for Pistachio:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	18145	**76**	6,11	**5**	AQ	
10	>4000		16,6	**15**	AQ	Mean calculated by DLA
24	27200	**114**	14	**12**	IL	
9	14923	**63**	10,7	**9**	NL	

RA*	50-150 %	RA*	50-150 %
Number in RA	**3**	Number in RA	**0**
Percent in RA	**100**	Percent in RA	**0**

* Range of acceptance of AOAC for allergen ELISAs

Methods:
AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = NutriLinia, Transia

Comments:
For the spiking material sample 3 participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the baked sample A produced with the spiking material sample none of the recovery rates were in the range of acceptance.

4.2.2 PCR-Results: Pistachio

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	positive		negative		2/2 (100%)	MS	
23	positive	194	negative		2/2 (100%)	MS	Mean calculated by DLA
5	positive	152	negative		2/2 (100%)	PL	
1	positive	> 0,4	negative	< 0,4	2/2 (100%)	SFA	
8	positive	≤ 0,4	negative	≤ 0,4	2/2 (100%)	SFA	
11	positive		positive		1/2 (50%)*	SFA	
13	positive		negative		2/2 (100%)	SFA	
17	positive		negative		2/2 (100%)	SFA	
21	positive		negative		2/2 (100%)	SFA	
25	positive	10,4	negative	-	2/2 (100%)	SFA	
7	positive		negative		2/2 (100%)	div	
16	positive		positive		1/2 (50%)*	div	
18	positive		positive	traces	1/2 (50%)*	div	
22	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	14		3	
Number negative	0		11	
Percent positive	100		21	
Percent negative	0		79	
Consensus	positive		negative	

Methods:

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

Comments:

There were 100% positive results for sample A and 79% negative results for sample B by the PCR-methods. The results are in qualitative agreement with the spiking of sample A.
Despite the consensus was "negative" for sample B, positive results for sample B in the range of the limits of detection / determination could not be valuated as "false-positive", because the occurrance of traces of pistachio in the matrix baking mixture could not be excluded.

Quantitative valuation of results: Sample A

Because of the low number of the quantitative results an statistical evaluation was not done.

Recovery Rates for Pistachio:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
14					MS	
23	94543	584	194	259	MS	Mean calculated by DLA
5	> LOQ		152	203	PL	
1	> 0,4		> 0,4		SFA	
8	≤ 0,4		≤ 0,4		SFA	
11					SFA	
13					SFA	
17					SFA	
21					SFA	
25	-		10,4	14	SFA	
7					div	
16					div	
18					div	
22					div	

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

* Range of acceptance of AOAC for allergen ELISAs

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

Comments:
None of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150% for the spiking material sample or for the baked sample A produced with the spiking material sample.

5. Documentation

Details by the participants

5.1 ELISA: Peanut

Primary data

Evaluation number	Result Sample A		Result Sample B		Result Spiking Sample		quantitative Result given as	Meth. Abr.	Method
	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
12	positive	50,0	negative	< 1	positive	31425	Peanut protein	AQ	AgraQuant F.A.S.T. Peanut (COKAL0148F), RomerLabs
4	positive	>30	negativee	<1	-		Peanut	BC	Peanut-Check ELISA, Bio-Check
3	positive	39	negative	<1	positive	7000	Peanut	BK	BioKits Peanut Assay Kit, Neogen
19	-	40	-	< 0,1	-		Peanut	BK	BioKits Peanut Assay Kit, Neogen
13	positive		negative		positive			ES	ELISA-Systems, Peanut Residue Assay (ESPRDT-48)
18	positive	75	negative		positive	98	Peanut	IL	Immunolab Peanut ELISA
24	positive	251	negative	< 1	positive	139000	Peanut	IL	Immunolab Peanut ELISA
1	positive	120	negativee	< 1,5	positive	49000	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
2	positive	87,52	negativee	<2.5	positive	50407	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
8	positive	143	negative	≤ 2,5	positive	95010	Peanut	RS	RIDASCREEN FAST Peanut, r-biopharm R6202
9	positive	111,6	negative		positive	52809	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
10	positive	111,5	negative	<LOD	-	>5000	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
11	positive	95,5	negativee	<1.5ppm	positive	>4000ppm	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
17	positive	106	negative	<2,5	positive	37685	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
20	positive	99,5	negative	<2,5	positive	39205	Peanut	RS	Ridascreen Fast Peanut (R6202), r-Biopharm
15	positive	101	negative	< 2.5	positive	> 500	Peanut	VT	Veratox Peanut Allergen, Neogen

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck
BK = Biokits, Neogen
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	
12	AQ	Peanut protein	according to testkit manual	2 results submitted each: Sample A 47,9 and 52,1 mg/kg and Spiking material sample 31800 and 31050 mg/kg
4	BC	Polyclonal	Aqueous Buffered Extraction 15min @ 60ºC	Spiking material not quantifiable due to assay range
3	BK		according to kit manual	
19	BK	polyclonal Ab against Conarachin (Ara h1)	according to kit manual	
13	ES		according to kit manual	
18	IL		according to kit manual	
24	IL			
1	RS			
2	RS	Peanut Proteins including Ara h1 & Ara h2	according to kit manual	1 in 10 dilution performed to obtain results within the range of the kit standards
8	RS		according to kit	
9	RS	Peanut protein	according to kit manual with milk powder	
10	RS			Single results Sample A 111,5 and 97,2 mg/kg
11	RS			
17	RS			
20	RS		according to kit manual	2 results submitted each: Sample A 102 and 97 mg/kg and Spiking material sample 37584 and 40825 mg/kg
15	VT			

5.2 ELISA: Pistachio

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
2	positive	6,11	negative	<1	positive	18145	Pistachio	AQ	AgraQuant Pistachio (COKAL2748), RomerLabs
10	positive	16,6	negative	<LOD	positive	>4000	Pistachio	AQ	AgraQuant Pistachio (COKAL2748), RomerLabs
24	positive	14	negative	< 1	positive	27200	Pistachio	IL	Immunolab Pistachio ELISA
9	positive	10,7	negative		positive	14923	Pistachio	NL	nutriLinia, Transia

Methods:

AQ = AgraQuant, RomerLabs
IL = Immunolab
NL = NutriLinia, Transia

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	
2	AQ	Pistachio Proteins	As per Kit Instructions	
10	AQ			Singel results: Sample A 18,6 and 14,5 mg/kg
24	IL			
9	NL			

5.3 PCR: Peanut

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
7	positive		negative		positive		Peanut-DNA	ASU	ASU §64 L 44.00-11 (PCR-Peanut)
9	positive		negative		positive		Peanut-DNA	ASU	ASU §64 L 44.00-11 (PCR-Peanut)
14	positive		negative		positive		Peanut-DNA	ASU	ASU §64 L 44.00-11 (PCR-Peanut)
18	positive		negative		positive		Peanut-DNA	ASU	ASU §64 L 44.00-11 (PCR-Peanut)
19	positive		negative		-		Peanut	ASU	5xQuantiFast® Pathogen PCR Fa.Qiagen (211354) Primer/Sonde:eurofins/mwg/operon,ASU L44.00-11
23	positive	728	negative		positive	269468	Peanut-DNA	MS	Köppel et al. 2012 (AllAll C, D)
5	positive	389	negative		positive	> LOQ	Peanut-DNA	PL	PLANTON GmbH; pmPES
6	negative		positive		positive			QG	QIAGEN
1	positive	> 10	negative	< 10	positive	> 10	Peanut-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
11	positive		negative		positive		Peanut-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
12	positive	51,88	negative	< 1	positive	27141,58	Peanut	SFA	Sure Food Allergen , Congen / r-Biopharm
13	positive		negative		positive			SFA	Sure Food Allergen , Congen / r-Biopharm
21	positive		negative		positive		Peanut-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
25	positive	-	negative	-	-	-	Peanut	SFA	Sure Food Allergen , Congen / r-Biopharm S3402 SureFood® ALLERGEN 4plex Peanut/Hazelnut/Walnut+IACSure Food Allergen , Congen / r-Biopharm S3402 SureFood® ALLERGEN 4plex Peanut/Hazelnut/Walnut+IAC
25	positive	68,8	negative	-	-	-	Peanut	SFA*	Sure Food Allergen , Congen / r-Biopharm S3203 SureFood® ALLERGEN QUANT Peanut Sure Food Allergen , Congen / r-Biopharm S3203 SureFood® ALLERGEN QUANT Peanut
7	positive		negative		positive		Peanut-DNA	div	Hird et al. Eur Food Res Technol (2003) 217:265–268+ Korrektur 2005
22	positive		negative		positive			div	in-house method

Methods:

ASU = ASU L 44.00-11
MS = AllAll C u. D, Microsynth
PL = Planton
QG = Qiagen
SFA = Sure Food Allergen S3402, R-Biopharm / Congen
SFA* = Sure Food Allergen S3202, S3203, 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	
7	ASU	Ara h 2 gen	Extraction §64 L 08.00-59	LOQ 100 mg/kg
9	ASU			
14	ASU	Peanut	Wizard/ Real Time PCR	
18	ASU		DNA Extraction with MN Food Kit plus Rnase/ Real Time PCR with 45 cycles	
19	ASU	86bp long sequence part from genes of Ara h2	Dneasy Rmericon Food Kit/ Proteinase K/ Real Time PCR/ 45 cycles	
23	MS	Peanut	Wizard Extraktion / Rotorgene / 45 cycles	2 results each: Sample A 765 and 691mg/kg and Spiking material sample 243260 and 295676 mg/kg
5	PL	s. SOP	PLANTON GmbH; CTAB; Magnetic Beads	
6	QG	Peanut agglutinin precursor	Dneasy Plant Mini Kit, PCR and Gel-electrophoresis	
1	SFA			
11	SFA			
12	SFA	Peanut	Extraction with Kit Congen SureFood® Prep Allergen; Real Time PCR; 45 cycles	according to manual LOD: 1 ppm; 2 results each: Sample A 48,58 and 55,17 mg/kg and Spiking material sample 25035,77 and 29247,38 mg/kg
13	SFA		SureFood PREP Allergen	
21	SFA	Peanut	CTAB /QIAquick / Real-Time PCR / 35 Zyklen	
25	SFA	Peanut	Limit of detection 1 mg/kg DNA-Exraction with SureFood® PREP Advanced Protocol 1	-
25	SFA*	Peanut	Limit of detection 1 mg/kg; Limit of quantification 4 mg/kg DNA-Exraction mit SureFood® PREP Advanced Protokoll 1	-
7	div	Ara h 2 gene	Extraction §64 L 08.00-59	LOQ 100 mg/kg
22	div	Peanut, <100bp DNA-fragment	2g Sample / Machery & Nagl Nucleospin Food / Sybr-Green / Real-Time PCR / 45 cycles	

5.4 PCR: Pistachio

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	positive		negative		positive		Pistachio-DNA	MS	Microsynth
23	positive	194	negative		positive	94543	Pistachio-DNA	MS	Köppel et al. 2012 (AllAll C, D)
5	positive	152	negative		positive	> LOQ	Pistachio-DNA	PL	PLANTON GmbH; [5-Pis]-Fam
1	positive	> 0,4	negative	< 0,4	positive	> 0,4	Pistachio-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
8	positive	≤ 0,4	negative	≤ 0,4	positive	≤ 0,4	DNA	SFA	SureFood ALLERGEN Pistachio, r-biopharm S3114
11	positive		positive		positive		Pistachio-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
13	positive		negative		positive			SFA	Sure Food Allergen , Congen / r-Biopharm
17	positive		negative		positive		Pistazie	SFA	Sure Food Allergen , Congen / r-Biopharm
21	positive		negative		positive		Pistachio-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
25	positive	10,4	negative	-	-	-	Pistazie	SFA	Sure Food Allergen , Congen / r-Biopharm S3214 SureFood®ALLERGEN QUANT PistachioSure Food Allergen , Congen / r-Biopharm S3214 SureFood®ALLERGEN QUANT Pistachio
7	positive		negative		positive		Pistachio-DNA	div	Brezna et al. Eur Food Res Technol (2008) 228: 197-203
16	positive		positive		positive			div	in-house method
18	positive		positive	traces	positive		Pistachio-DNA	div	Literature Brezna et al
22	positive		negative		positive			div	in-house method

Methods:

MS = AllAll, Microsynth
PL = Planton
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	MS	Pistachio	Wizard/ Real Time PCR	
23	MS	Pistachio	Wizard Extraction / Rotorgene / 45 cycles	2 results each: Sample A 243 and 145 mg/kg and Spiking material sample 78678 and 110408 mg/kg
5	PL	s. SOP	PLANTON GmbH; CTAB; Magnetic Beads	
1	SFA			
8	SFA		DNA extraction according to foodproof Magnetic	
11	SFA			
13	SFA		SureFood PREP Allergen	
17	SFA			
21	SFA	Pistachio	CTAB /QIAquick / Real-Time PCR / 35 cycles	
25	SFA	Pistachio	Limit of detection 1 mg/kg; Limit of quantification 4 mg/kg; DNA-Exraction mit SureFood® PREP Advanced Protokoll 1	-
7	div	mRNA for dehydrin (COR gene) Y07600	Extraction §64 L 08.00-59	Cut Off: 10 mg/kg; Traces Pistachio-DNA in Sample B
16	div			Sample B: weakly positive; in the range of LOD
18	div		DNA Extraction with MN Food Kit plus Rnase/ Real Time PCR with 45 cycles	
22	div	Pistachio, 90bp DNA-fragment	2g Sample / Machery & Nagl Nucleospin Food / Sybr-Green / Real-Time PCR / 45 cycles	

6. Index of participant laboratories

Teilnehmer /Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		GERMANY
		FRANCE
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		ITALY
		AUSTRIA
		GERMANY
		GERMANY
		SWITZERLAND
		FRANCE
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GREAT BRITAIN
		THAILAND
		GERMANY
		GERMANY
		NETHERLANDS

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

7. Index of references

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