DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

06/2014

Allergens VI:

Hazelnut and Almond

in Chocolate

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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 material is a common in commerce dark chocolate. The basic composition of both sample A and sample B was the same (see table 1). Before sample A was spiked the chocolate was tempered to 60°C. Then while stirring the spiking material sample containing hazelnut and almond was added, mixed and diluted with additional chocolate by several steps. Then the samples were packaged in portions to approximately 20 g.

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

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Dark-Chocolate **(cacao: 70% at least)** Ingredients: Cocoa mass, sugar, cacoa butter, emulsifier: soy lecithin, vanilla extract Nutrients per 100 g: Protein 7,7 g, carbohydrates 34 g, fat 42 g Allergen-Information: may contain traces of peanuts, almonds, nuts and milk.	100 g/100 g	100 g/100 g
Spiking material sample	0,45 g/100g	-

Table 2: Added amounts of allergenic ingredients

Ingredients	**Spiking material sample**	**Content in Sample A**
Potato flour	81 %	0,37 %
Hazelnut spread Ingredients: Sugar, hazelnut (25%), vegetable oils, lactose, skimmed milk powder (5%), defatted cocoa powder (2%), emulsifier: lecithins	62300 mg/kg (6,23 %)	280 mg/kg
- as Hazelnut thereof Hazelnutprotein	15600 mg/kg 1560 mg/kg	70 mg/kg 7,0 mg/kg
Almond mush, white Ingredients: Sweet Almonds Nutrients per 100g: Protein 23 g	20400 mg/kg (2,04 %)	92 mg/kg
- as Almond - thereof Almondprotein	20400 mg/kg 4690 mg/kg	92 mg/kg 21 mg/kg
Peanut mush	2,59 %	0,012 %
Pistachio spread	7,95 %	0,036 %

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).

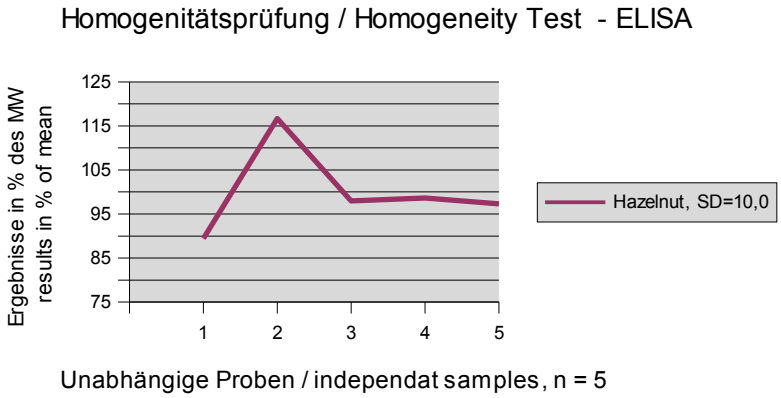

Fig. 1: Testing of homogeneity of DLA-sample A
Results are given in percent of the arithmetic mean
(tested for almond content)

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 42nd week of 2014. The testing method was optional. The tests should be finished at November 28th 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. hazelnut or almond in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specifity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.
One participant submitted no results, 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 hazelnut protein or almond protein were converted to hazelnut and almond. When possible the information supplied by the test kit manufacturer was used. A protein content of 10% for hazelnut and 25% for almond 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 Hazelnut

4.1.1 ELISA-Results: Hazelnut

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		
9	positive	10,8	negative	< 1	2/2 (100%)	AQ1	
14	positive	25,8	negative	< 2	2/2 (100%)	AQ2	mean calculated by DLA
5	positive	26	negative	< 0.5	2/2 (100%)	ES	result converted
15	positive	18	negative	< 0.5	2/2 (100%)	ES	result converted
16	positive	38	negative	< 0,5	2/2 (100%)	ES	result converted
1	positive	14,68	negative	< 2.5	2/2 (100%)	RS	
4	positive	24,4	negative	< 1,5	2/2 (100%)	RS	
6	positive	17,38	negative	< 2,5	2/2 (100%)	RS	
7	positive	18	negative	< 1,5	2/2 (100%)	RS	
8	positive	25,5	negative	< 2,5	2/2 (100%)	RS	
10	positive	11	negative	< 1.5	2/2 (100%)	RS	
11	positive	23,3	negative	-	2/2 (100%)	RS	
12	positive	25	negative	< 2,5	2/2 (100%)	RS	
13	positive	20,3	negative	< 2,5	2/2 (100%)	RS	
17	positive	13,1	negative		2/2 (100%)	RS	
19	positive	12,9	negative	< 2,5	2/2 (100%)	RS	mean calculated by DLA
20	positive	16,2	negative	< NG	2/2 (100%)	RS	
22	positive	11,02	negative	< 2,50	2/2 (100%)	RS	mean calculated by DLA
2	positive	28,6	negative	< 0.5	2/2 (100%)	VT	

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

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
ES = ELISA Systems
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	Hazelnut	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
9	10,8	-1,8		AQ1	
14	25,8	1,3		AQ2	mean calculated by DLA
5	26	1,3		ES	result converted
15	18	-0,3		ES	result converted
16	38	3,8		ES	outlier X_{all}, result converted
1	14,68	-1,0	-0,7	RS	
4	24,4	1,0	1,4	RS	
6	17,38	-0,5	-0,1	RS	
7	18	-0,3	0,0	RS	
8	25,5	1,2	1,7	RS	
10	11	-1,8	-1,5	RS	
11	23,3	0,8	1,2	RS	
12	25	1,1	1,6	RS	
13	20,3	0,1	0,5	RS	
17	13,1	-1,3	-1,1	RS	
19	12,9	-1,4	-1,1	RS	mean calculated by DLA
20	16,2	-0,7	-0,4	RS	
22	11,02	-1,8	-1,5	RS	mean calculated by DLA
2	28,6	1,8		VT	

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
ES = ELISA Systems
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Characteristics: Quantitative evaluation Hazelnut

Sample A

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*19*	*13*
Robust mean (X)	19,6	17,9
Robust standard deviation (S^x)	7,26	6,05
Median	18	17,4
Target range:		
Target standard deviation ($\hat{\sigma}$)	4,90	4,48
lower limit of target range (X - 2 $\hat{\sigma}$)	9,80	8,96
upper limit of target range (X + 2 $\hat{\sigma}$)	29,4	26,9
Quotient $S^x/\hat{\sigma}$	*1,5*	*1,4*
Standard uncertainty u_X	*2,1*	*2,1*
Quotient $u_X/\hat{\sigma}$	*0,42*	*0,47*
Number of results in the target range	*18 (95%)*	*13 (100%)*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments:
The evaluation of all methods and the evaluation of results from method RS showed a normal 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 28% and 26% of the spiking level (s. "Recovery rates of Hazelnut" p.18).

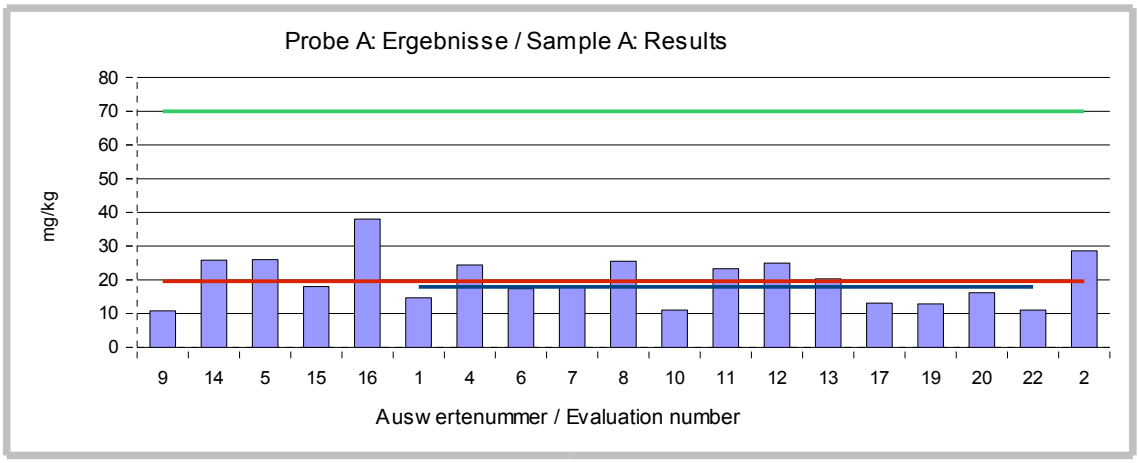

Fig. 2: ELISA-Results Hazelnut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

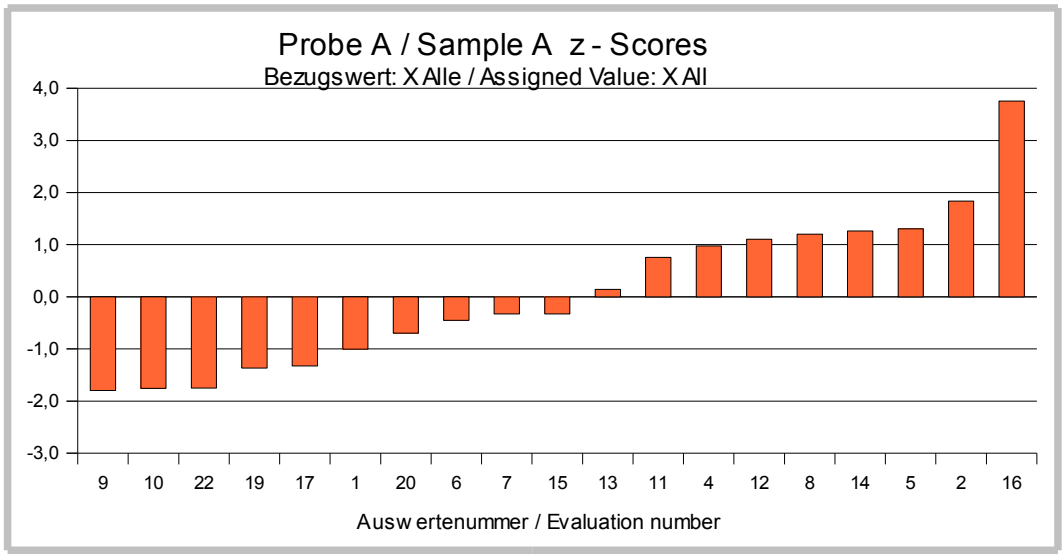

Fig. 3: z-Scores (ELISA-Results as Hazelnut) Assigned value robust mean of all results

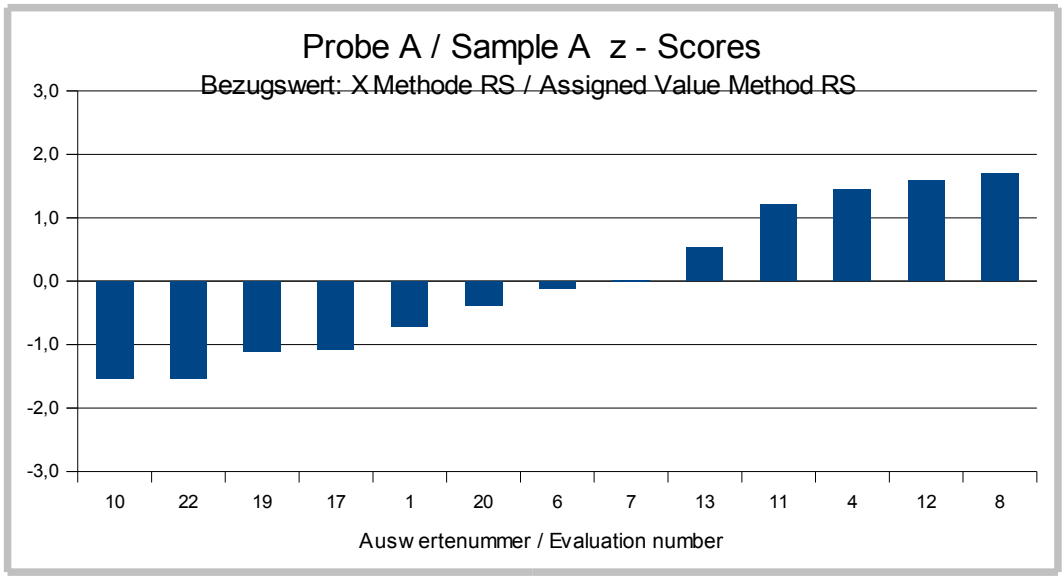

Fig. 4: z-Scores (ELISA-Results as Hazelnut) Assigned value robust mean of method RS (R-Biopharm, Ridascreen Fast)

Recovery Rates for Hazelnut:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
9	6603	42	10,8	15	AQ1	
14	6130	39	25,8	37	AQ2	mean calculated by DLA
5	7200	46	26	37	ES	result converted
15	> 500		18	26	ES	result converted
16	9600	**62**	38	**54**	ES	result converted
1	4389	28	14,68	21	RS	
4			24,4	35	RS	
6	4239,96	27	17,38	25	RS	
7	4600	29	18	26	RS	
8	6532	42	25,5	36	RS	
10	856	5	11	16	RS	
11	-		23,3	33	RS	
12	5800	37	25	36	RS	
13	3594	23	20,3	29	RS	
17	4500	29	13,1	19	RS	
19	3870	25	12,9	18	RS	mean calculated by DLA
20	7000	45	16,2	23	RS	spiking material sample indicated as „approximately“
22	5728,8	37	11,02	16	RS	mean calculated by DLA
2	>10000		28,6	41	VT	

RA*	50-150 %	RA*	50-150 %
Number in RA	**1**	Number in RA	**1**
Percent in RA	**7**	Percent in RA	**5**

* Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
ES = ELISA Systems
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 chocolate sample A produced with the spiking material sample also only one recovery rate was in the range of acceptance.

4.1.2 PCR-Results: Hazelnut

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		
21a	positive		negative		2/2 (100%)	ASU	
3	positive	-	negative	-	2/2 (100%)	MS/Kö	
18	positive	<100	negative		2/2 (100%)	MS/Kö	
7	positive	> 10	negative	< 10	2/2 (100%)	SFA-1	
10	positive		negative		2/2 (100%)	SFA-1	
11a	positive	33,1	negative	-	2/2 (100%)	SFA-1	
14	positive	42,4	negative	< 1	2/2 (100%)	SFA-1	Mittelwert von DLA berechnet
11b	positive	-	negative	-	2/2 (100%)	SFA-2	
12	positive		negative		2/2 (100%)	div	
21b	positive		negative		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	10		0	
Number negative	0		10	
Percent positive	100		0	
Percent negative	0		100	
Consensus	positive		negative	

Methods:

ASU = ASU L 44.00-8
MS/Kö = AllAll C u. D, Microsynth / Köppel et al. 2012
SFA-1 = Sure Food Allergen S3202, R-Biopharm / Congen
SFA-2 = Sure Food Allergen S3402 4plex, R-Biopharm / Congen
div = not indicated / other method

Comments:

For the detection of hazelnut by PCR 100% positive results for sample A and 100% negative results for sample B were obtained. 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 Hazelnut:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample B	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
21a					ASU	
3			-		MS/Kö	
18	93000	596	<100		MS/Kö	
7	> 10		> 10		SFA-1	
10					SFA-1	
11a	-		33,1	47	SFA-1	
14	5195	33	42,4	**61**	SFA-1	mean calculated by DLA
11b	-		-		SFA-2	
12					div	
21b					div	

RA*	50-150 %	RA*	50-150 %
Number in RA	**0**	Number in RA	**1**
Percent in RA	**0**	Percent in RA	**50**

* Range of acceptance of AOAC for allergen ELISAs

Methods:
ASU = ASU L 44.00-8
MS/Kö = AllAll C u. D, Microsynth / Köppel et al. 2012
SFA-1 = Sure Food Allergen S3202, R-Biopharm / Congen
SFA-2 = Sure Food Allergen S3402 4plex, R-Biopharm / Congen
div = not indicated / other method

Comments:
For the spiking material sample none of the participants' results obtained a recovery rate in the range of 50-150% using PCR. For the chocolate-sample A spiked with the spiking material sample one of the recovery rates was in the range of acceptance.

4.2 Proficiency Test Almond

4.2.1 ELISA-Results: Almond

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		
9	positiv	67,7	negativ	< 0.4	2/2 (100%)	AQ1	
14	positiv	98,1	negativ	< 1	2/2 (100%)	AQ2	mean calculated by DLA
15	positiv	> 10	negativ	< 0.5	2/2 (100%)	NL	
1	positiv	89,28	negativ	< 2.5	2/2 (100%)	RS	
4	positiv	85,5	negativ	<1,7	2/2 (100%)	RS	
5	positiv	78,8	negativ	< 2.5	2/2 (100%)	RS	
6	positiv	89,4	negativ	< 2,5	2/2 (100%)	RS	
7	positiv	74	negativ	< 1,7	2/2 (100%)	RS	
8	positiv	102	negativ	< 2,5	2/2 (100%)	RS	
10	positiv	25,2	negativ	< 1.7	2/2 (100%)	RS	
13	positiv	62	negativ	< 2,5	2/2 (100%)	RS	
16	positiv	98	negativ	< 2,5	2/2 (100%)	RS	
17	positiv	86,4	negativ		2/2 (100%)	RS	
19	positiv	67,0	negativ	< 2,5	2/2 (100%)	RS	mean calculated by DLA
20	positiv	328	negativ	< LOD	2/2 (100%)	RS	result converted
22	positiv	75,0	negativ	<2,50	2/2 (100%)	RS	mean calculated by DLA
2	positiv	59,8	negativ	< 0.5	2/2 (100%)	VT	

	Sample A		Sample B	
Number positive	17		0	
Number negative	0		17	
Percent positive	100		0	
Percent negative	0		100	
Consensus	positiv		negativ	

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
NL = NutriLinia, Transia
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

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 evaluation of results: Sample A

Evaluation number	Almond	z-Score X_{ALL}	z-Score X_{RS}	Method	Remarks
	[mg/kg]	X_{ALL}	$X_{Method\ RS}$		
9	67,7	-0,7		AQ1	
14	98,1	0,9		AQ2	mean calculated by DLA
15	> 10			NL	
1	89,28	0,4	0,3	RS	
4	85,5	0,2	0,1	RS	
5	78,8	-0,1	-0,2	RS	
6	89,4	0,4	0,3	RS	
7	74	-0,3	-0,4	RS	
8	102	1,0	0,9	RS	
10	25,2	-2,8	-2,8	RS	
13	62	-0,9	-1,0	RS	
16	98	0,8	0,8	RS	
17	86,4	0,3	0,2	RS	
19	67,0	-0,7	-0,8	RS	mean calculated by DLA
20	328	12,2	11,9	RS	outlier X_{All} a. $X_{RS,\ result\ converted}$
22	75,0	-0,3	-0,4	RS	mean calculated by DLA
2	59,8	-1,0		VT	

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
NL = NutriLinia, Transia
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Characteristics: Quantitative evaluation Almond

Sample A

	All Results *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value	X_{ALL}	$X_{Method\ RS}$
Number of results	*16*	*13*
Robust mean (X)	80,9	82,5
Robust standard deviation (S^x)	16,6	17,9
Median	82,2	85,5
Target range:		
Target standard deviation ($\hat{\sigma}$)	20,2	20,6
lower limit of target range (X - 2 $\hat{\sigma}$)	40,5	41,3
upper limit of target range (X + 2 $\hat{\sigma}$)	121	124
Quotient $S^x/\hat{\sigma}$	*0,82*	*0,87*
Standard uncertainty u_X	*5,2*	*6,2*
Quotient $u_X/\hat{\sigma}$	*0,26*	*0,30*
Number of results in the target range	*14 (88%)*	*11 (85%)*

Method:
RS = R-Biopharm, Ridascreen Fast®

Comments:
The evaluation of all methods and the evaluation of results from method RS showed a low variability, respectively. The quotients $S^x/\hat{\sigma}$ were below 1,0. The comparability of results is given.
The mean of the evaluation was about 88% and 90% of the spiking level (s. "Recovery rates of Almond" p.26).

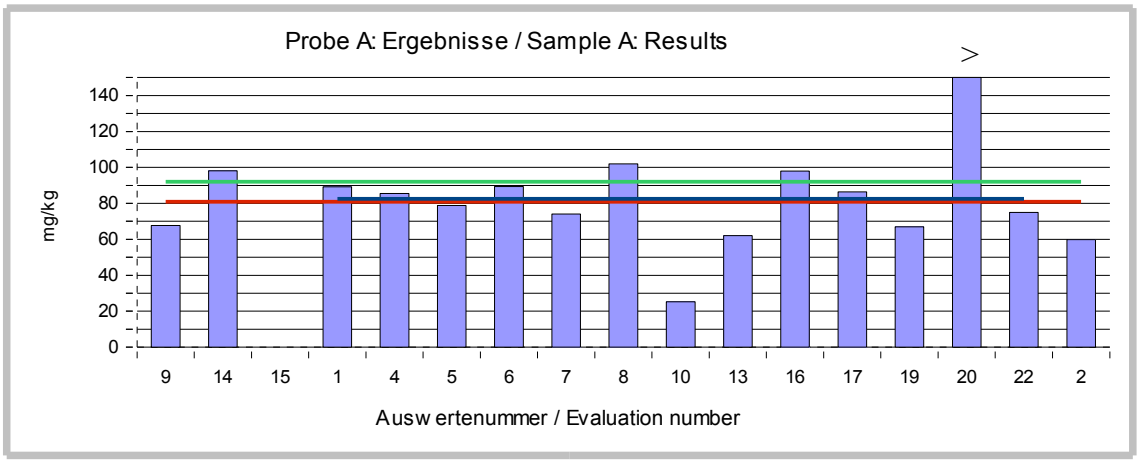

Fig. 5: ELISA-Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS

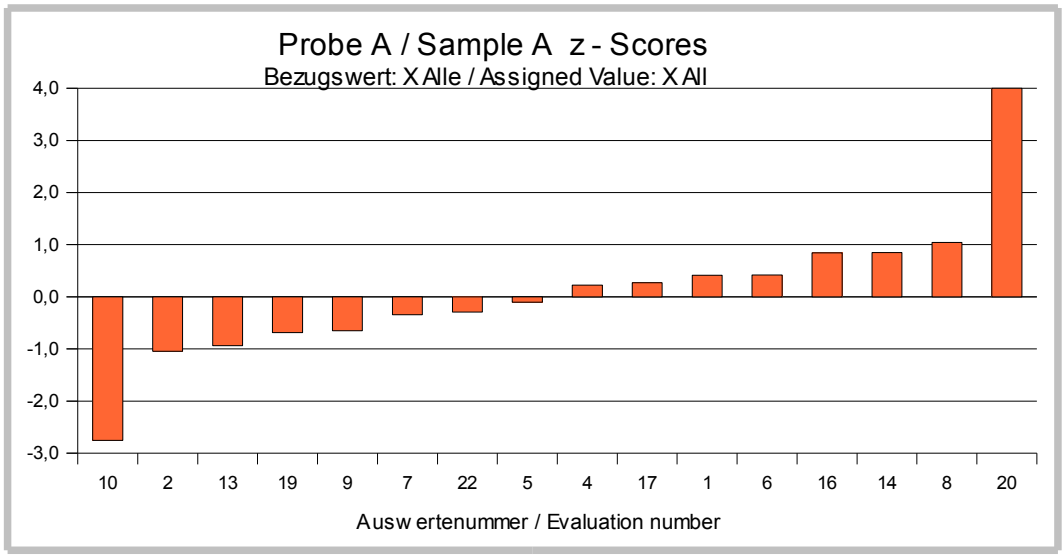

Fig. 6: z-Scores (ELISA-Results as Almond) Assigned value robust mean of all results

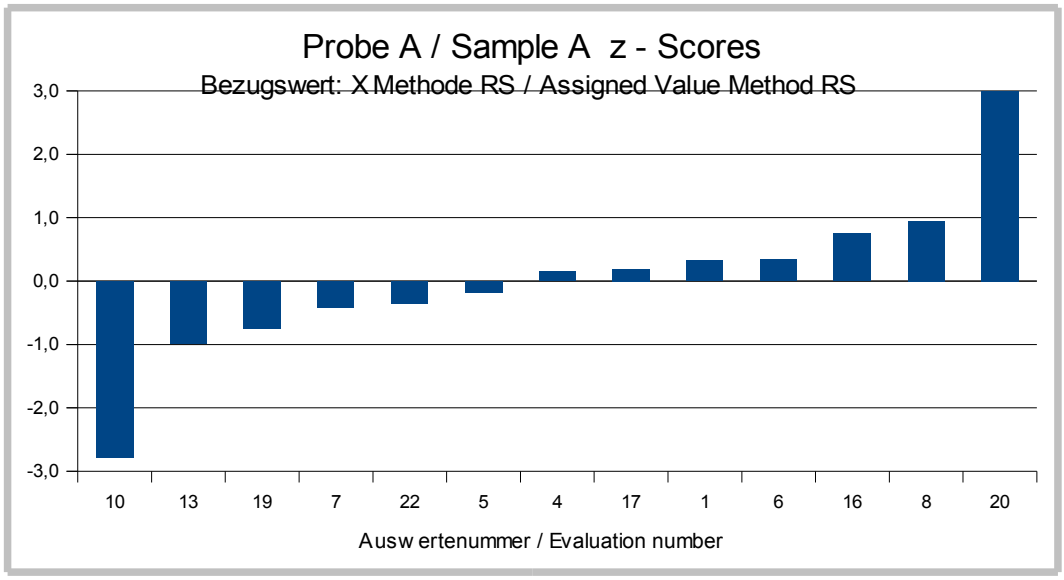

Fig. 7: z-Scores (ELISA-Results as Almond) Assigned value robust mean of method RS (R-Biopharm, Ridascreen Fast)

Recovery Rates for Almond:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
9	17580	**86**	67,7	**74**	AQ1	
14	10050	49	98,1	**107**	AQ2	mean calculated by DLA
15			> 10		NL	
1	17051	**84**	89,28	**97**	RS	
4			85,5	**93**	RS	
5	14000	**69**	78,8	**86**	RS	
6	26021,5	**128**	89,4	**97**	RS	
7	17000	**83**	74	**80**	RS	
8	22345	**110**	102	**111**	RS	
10	> 20		25,2	27	RS	
13	13171	**65**	62	**67**	RS	
16	25000	**123**	98	**107**	RS	
17	26000	**127**	86,4	**94**	RS	
19	19934	**98**	67,0	**73**	RS	mean calculated by DLA
20	80000	392	328	357	RS	result converted, spiking material sample indicated as „approximately“
22	>10000		75,0	**82**	RS	mean calculated by DLA
2	>10000		59,8	**65**	VT	

RA*	50-150 %	RA*	50-150 %
Number in RA	**10**	Number in RA	**14**
Percent in RA	**83**	Percent in RA	**88**

* Range of acceptance of AOAC for allergen ELISAs

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
NL = NutriLinia, Transia
RS = R-Biopharm, Ridascreen®
VT = Veratox, Neogen

Comments:

For the spiking material sample 83% of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the chocolate-sample A produced with the spiking material sample 88% of the recovery rates were in the range of acceptance.

4.2.2 PCR-Results: Almond

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		
3	positive	-	negative	-	2/2 (100%)	MS/Kö	
17a	positive	92,5	negative		2/2 (100%)	MS/Kö	
17b	positive	110	negative		2/2 (100%)	MS/Kö	
18	positive	<100	negative		2/2 (100%)	MS/Kö	
7	positive	> 5	negative	< 5	2/2 (100%)	SFA	
10	positive		negative		2/2 (100%)	SFA	
11	positive	-	negative	-	2/2 (100%)	SFA	
14	positive	na	negative	< 4	2/2 (100%)	SFA	
12	positive		negative		2/2 (100%)	div	
16	positive	-	negative	-	2/2 (100%)	div	
21	positive		negative		2/2 (100%)	div	

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

Methods:

MS/Kö = AllAll C u. D, Microsynth / Köppel et al. 2012
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:

For the detection of almond by PCR 100% positive results for sample A and 100% negative results for sample B were obtained. 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 Almond:
Spiking Material Sample and Sample A

Evaluation number	Spiking material	Recovery rate	Sample A	Recovery rate	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3			-		MS/Kö	
17a	27380	**134**	92,5	**101**	MS/Kö	
17b	4830	24	110	**120**	MS/Kö	
18	92000	451	<100		MS/Kö	
7	> 5		> 5		SFA	
10					SFA	
11	-		-		SFA	
14	na		na		SFA	
12					div	
16	-		-		div	
21					div	

RA*	50-150 %	RA*	50-150 %
Number in RA	**1**	Number in RA	**2**
Percent in RA	**33**	Percent in RA	**100**

* Range of acceptance of AOAC for allergen ELISAs

Methods:
MS/Kö = AllAll C u. D bzw. B u. G, Microsynth / Köppel et al. 2012
SFA = Sure Food Allergen, R-Biopharm / Congen
div = not indicated / other method

Comments:
One of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150% for the spiking material sample and two participants for the chocolate-sample A produced with the spiking material sample.

5. Documentation

Details by the participants

5.1 ELISA: Hazelnut

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
9	positive	10,8	negative	< 1	positive	6603	Hazelnut	AQ1	AgraQuant Hazelnut (COKAL0348), RomerLabs
14	positive	25,4	negative	< 2	positive	6100	mg Hazelnut / kg food	AQ2	Agraquant F.A.S.T. Hazelnut (Code 4302075)
14	positive	26,1	negative	< 2	positive	6160	mg Hazelnut / kg food	AQ2	Agraquant F.A.S.T. Hazelnut (Code 4302075)
5	-	2,6	-	< 0.5	-	720	Hazelnutprotein	ES	ELISA-Systems, Hazelnut Residue Assay (ESHRD-48)
15	positive	1,8	negative	< 0.5	positive	> 50	Hazelnutprotein	ES	ELISA-Systems, Hazelnut Residue Assay (ESHRD-48)
16	positive	3,8	negative	< 0,5	positive	960	Hazelnutprotein	ES	ELISA-Systems, Hazelnut Residue Assay (ESHRD-48)
1	positive	14,68	negative	< 2.5	positive	4389	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
4	positive	24,4	negative	< 1,5	positive		Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
6	-	17,38	-	< 2,5	-	4239,96	Hazelnut	RS	RIDASCREEN FAST Hazelnut, r-biopharm R6802
7	positive	18	negative	< 1,5	positive	4600	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
8	positive	25,5	negative	< 2,5	positive	6532	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
10	positive	11	negative	< 1.5	positive	856	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
11	positive	23,3	negative	-	-	-	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
12	positive	25	negative	< 2,5	positive	5800	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
13	-	20,3	-	< 2,5	-	3594	Hazelnut	RS	r-biopharm
17	positive	13,1	negative		positive	4500	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
19	positive	12,9	negative	< 2,5	positive	4420	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
19	positive	12,9	negative	< 2,5	positive	3326	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
20	-	16,2	-	< LOD	-	ca 7.000	Hazelnut part	RS	RIDASCREEN FAST Hazelnut r-Biopharm AG
22	positive	11,01	negative	< 2,50	positive	5956,1	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
22	positive	11,03	negative	< 2,50	positive	5501,4	Hazelnut	RS	Ridascreen Fast Hazelnut (R6802), r-Biopharm
2	positive	28,6	negative	< 0.5	positive	>10000	Hazelnut	VT	Veratox Hazelnut Allergen, Neogen

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
ES = ELISA Systems
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	
9	AQ1			
14	AQ2			
14	AQ2			
5	ES			
15	ES			
16	ES		according to kit manual	
1	RS	Hazelnut	As per Kit Instructions	Used 1 gram of skimmed milk powder for extraction
4	RS			
6	RS		according to kit	
7	RS			
8	RS		according to kit manual	
10	RS			
11	RS	-	limit of detection 1,5 mg/kg; limit of quantification 2,5 mg/kg	-
12	RS			quantitative
13	RS	Hazelnut	according to kit manual	Spiking: 1:500
17	RS		according to kit manual	
19	RS			
19	RS			
20	RS	not indicated	according to kit manual	
22	RS			Lot of Kit: 14374
22	RS			Lot of Kit: 14374
2	VT			

5.2 ELISA: Almond

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
9	positive	67,7	negative	< 0.4	positive	17580	Almond	AQ1	AgraQuant Almond (COKAL0748), RomerLabs
14	positive	100,7	negative	< 1	positive	11010	mg Almond / kg food	AQ2	Agraquant F.A.S.T. Almond (Code 4302072)
14	positive	95,4	negative	< 1	positive	9090	mg Almond / kg food	AQ2	Agraquant F.A.S.T. Almond (Code 4302072)
15	positive	> 10	negative	< 0.5	-		Almond	NL	nutriLinia Almond-E ELISA (NC-6018), Transia
1	positive	89,28	negative	< 2.5	positive	17051	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
4	positive	85,5	negative	<1,7	positive		Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
5	-	78,8	-	< 2.5	-	14000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
6	-	89,4	-	< 2,5	-	26021,5	Almond	RS	RIDASCREEN FAST Mandel/Almond, r-biopharm R6901
7	positive	74	negative	< 1,7	positive	17000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
8	positive	102	negative	< 2,5	positive	22345	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
10	positive	25,2	negative	< 1.7	positive	>20ppm	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
13	-	62	-	< 2,5	-	13171	Almond	RS	r-biopharm
16	positive	98	negative	< 2,5	positive	25000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
17	positive	86,4	negative		positive	26000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
19	positive	68,6	negative	< 2,5	positive	20000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
19	positive	65,4	negative	< 2,5	positive	19868	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
20P	-	82,0	-	< NG	-	ca 20.000	Almondprotein	RS	RIDASCREEN FAST Almond r-Biopharm AG
22	positive	77,28	negative	<2,50	positive	>10000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
22	positive	72,63	negative	< 2,50	positive	>10000	Almond	RS	Ridascreen Fast Almond (R6901), r-Biopharm
2	positive	59,8	negative	< 0.5	positive	>10000	Almond	VT	Veratox Almond Allergen, Neogen

Methods:

AQ1 = AgraQuant, RomerLabs
AQ2 = AgraQuant F.A.S.T., RomerLabs
NL = NutriLinia, Transia
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	
9	AQ1			
14	AQ2			
14	AQ2			
15	NL			
1	RS	Almond	As per Kit Instructions	Used 1 gram of skimmed milk powder for extraction
4	RS			
5	RS			
6	RS		according to kit	
7	RS			
8	RS		As per Kit Instructions	
10	RS			
13	RS	Almond	As per Kit Instructions	Spiking: 1:2500
16	RS		As per Kit Instructions	
17	RS		As per Kit Instructions	
19	RS			
19	RS			
20P	RS	polyclonal	As per Kit Instructions	
22	RS			Lot of Kit: 11394
22	RS			Lot of Kit: 11394
2	VT			

5.3 PCR: Hazelnut

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
21a	positive		negative		positive		Please select!	ASU	ASU §64 L 44.00-8 (PCR-Hazelnut)
3	positive	-	negative	-	-		Please select!	MS/Kö	Wizard Promega, Köppel et al. 2012
18	positive	<100	negative		positive	93000	Hazelnut-DNA	MS/Kö	Köppel et al. 2012 (AllAll C, D)
7	positive	> 10	negative	< 10	positive	> 10	Hazelnut-DNA	SFA-1	Sure Food Allergen , Congen / r-Biopharm
10	positive		negative		positive		Hazelnut-DNA	SFA-1	Sure Food Allergen , Congen / r-Biopharm
11a	positive	33,1	negative	-	-	-	Hazelnut	SFA-1	Sure Food Allergen , Congen / r-Biopharm S3202 SureFood®ALLERGEN QUANT Hazelnut
14	positive	42	negative	< 1	positive	5178	mg Hazelnut / kg food	SFA-1	SureFood Allergen Quant Hazelnut
14	positive	42,8	negative	< 1	positive	5212	mg Hazelnut / kg food	SFA-1	SureFood Allergen Quant Hazelnut
11b	positive	-	negative	-	-	-	Hazelnut	SFA-2	Sure Food Allergen , Congen / r-Biopharm S3402 SureFood®ALLERGEN 4plex Peanut/Hazelnut/Walnut+IAC
12	positive		negative		positive		Hazelnut-DNA	div	other: Köppel. R., et al., Eur Food Res Technol. 230 (2010)
21b	positive		negative		positive		Please select!	div	In-House Methode

Methods:

ASU = ASU L 44.00-8
MS/Kö = AllAll C u. D, Microsynth / Köppel et al. 2012
SFA-1 = Sure Food Allergen S3202, R-Biopharm / Congen
SFA-2 = Sure Food Allergen S3402 4plex, 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	
21a	ASU	Hazelnut	Macherey & Nagel, Nucleospin Food/Real Time PCR / 45 cycles	
3	MS/Kö			
18	MS/Kö	Hazelnut	Wizard extraction / Rotorgene / 45 cycles	Spiking sample: additional allergens detected: Peanut-DNA (837000 mg/kg), Cow-DNA (Milk) (8000 mg(kg), Pistachio-DNA (231000 mg/kg)
7	SFA-1			
10	SFA-1			
11a	SFA-1	Hazelnut	limit of detection 0,4 mg/kg; limit of quantification 1 mg/kg; DNA-Extraction with SureFood® PREP Advanced	-
14	SFA-1			
14	SFA-1			
11b	SFA-2	Hazelnut	limit of detection 1 mg/kg; DNA-Extraction with SureFood® PREP Advanced	-
12	div	Cor a 1 -gene	Extraction: Biotecon GMO sample preparation kit+alfa-amylase, +RNAase, Real-time PCR: Taqman, FAM-TAMRA, 45 cycles, Reference gene: actin	
21b	div	Hazelnut	Macherey & Nagel, Nucleospin Food/Real Time PCR / 45 cycles	

5.4 PCR: Almond

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	positive	-	negative	-			Please select!	MS/Kö	Wizard Promega, Köppel et al. 2012
17	positive	92,5	negative		positive	27380	Almond	MS/Kö	Microsynth
17	positive	110	negative		positive	4830	Almond	MS/Kö	Microsynth
18	positive	<100	negative		positive	92000	Almondn-DNA	MS/Kö	Köppel et al. 2012 (AllAll C, D)
7	positive	> 5	negative	< 5	positive	> 5	Almond-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
10	positive		negative		positive		Almond-DNA	SFA	Sure Food Allergen , Congen / r-Biopharm
11	positive	-	negative	-	-	-	Almond	SFA	Sure Food Allergen , Congen / r-Biopharm S3104 SureFood®ALLERGEN ID Almond
14	positive	na	negative	nd < 4	positive	na	Almond	SFA	SureFood Allergen Almond
12	positive		negative		positive		Almond-DNA	div	other: Köppel. R., et al., Eur Food Res Technol. 230 (2010)
16	positive	-	negative	-	positive	-	Almond-DNA	div	Selectionl PCR-Methods
21	positive		negative		positive		Please select!	div	In-House Methode

Methods:

MS/Kö = AllAll, Microsynth / Köppel et al. 2012
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	
3	MS/Kö			
17	MS/Kö	Almond	Extr mit Sure Food Prep Allergen, PCR with **AllAllB**	
17	MS/Kö	Almond	Extr mit Sure Food Prep Allergen, PCR with **AllAllG**	
18	MS/Kö	Almond	Wizard extraction / Rotorgene / 45 cycles	Spiking sample: additional allergens detected: Peanut-DNA (837000 mg/kg), Cow-DNA (Milk) (8000 mg(kg), Pistachio-DNA (231000 mg/kg)
7	SFA			
10	SFA			
11	SFA	Almond	limit of detection 4 mg/kg; DNA-Exraction with SureFood® PREP Advanced	-
14	SFA			
12	div	PRU A 1-gene	Extraction: Biotecon GMO sample preparation kit+alfa-amylase, +RNAase, Real-time PCR: Taqman, FAM-TAMRA, 45 cycles, Reference gene: actin	
16	div	Almond	DNA-Extraction: CTAB/Chloroform/Wizard Clean-Up + Realtime PCR 45 Cycles	
21	div	Almond	Macherey & Nagel, Nucleospin Food/Real Time PCR / 45 cycles	

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SWITZERLAND
		FRANCE
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
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		GREAT BRITAIN
		SPAIN
		GERMANY
		GREAT BRITAIN
		NETHERLANDS

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

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