Proficiency Tests
DLA
food
cosmetics
consumer goods
www.dla-lvu.de

Evaluation Report
proficiency test

DLA 17/2017

ALM Verification:

Gluten in Maize-Chips-Matrix

5 Samples containing Wheat Flour (Gluten Levels 2,0 / 10 / 20 / 50 / 100 mg/kg)

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

proficiency-testing@dla-lvu.de www.dla-lvu.de

Coordinator of this PT:
Dr. Matthias Besler

Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA 17/2017
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (27 July 2017) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler (Technischer Leiter / Technical Manager) - *gezeichnet / signed M. Besler* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - *gezeichnet / signed G. Wichmann* Datum / Date: 27 July 2017
Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Inhalt / Content

1. Introduction

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

The present PT-format **„Action Level Matrix - ALM Verification"** offers the possibility to prove that the analytical determination method applied by the participating laboratory is capable to reliably detect the allergen content relevant for food labelling by means of a kind of calibration row of 5 samples containing the allergen in a specific food-matrix and a blank sample.
The allergen contents of the PT-sample series vary from 1/10 to 2-fold of the action level, which is normally based on the threshold value dose (VITAL Concept 2.0) or the assessment values of the ALTS/ALS (German Food Expert Committee) (see Table 3). The evaluation of PT-results was performed qualitative in scores from 1-5 (Score 3 = Action Level successfully detected). Quantitative results were given including the recovery rate for information in the report.

2. Realisation

2.1 Test material

6 PT-samples with the food matrix Maize-Chips were provided for qualitative detection and optionally quantitative detection of gluten. The gluten-levels of the PT-sample series were in the range from 2 mg/kg to 100 mg/kg, whereas the medial level represents the "Action Level" (see Table 1).
The food matrix of sample material was common in commerce Maize-Chips (declaration as gluten free). The basic composition was identical for all 6 samples (see Table 1).
After crushing and sieving using an impact mill (mesh 3,0 mm) the basic mixture was homogenized and an aliquot was taken from it as blank sample.

For preparation of the gluten containing samples Maize-Chips were baked (195°C, 15 min) and dried (60°C, 3h) using Maize flour and a wheat-flour-mixture (further information see below). Afterwards the gluten-Maize-Chips were crushed by a centrifugal mill (mesh< 250 µm) and homogenized.

Afterwards the **spiked sample series** was produced as follows: After crushing and homogenization an aliquot of the gluten containing Maize-Chips was added to the basic mixture. The resulting mixture was homogenized again. Afterwards basic mixture was added stepwise (3-5 steps) including mechanical homogenization after each step until the total amount of sample material was reached.

The 6 PT-samples were portioned to approximately 20 g in metallized PET film bags.

For the spiking a wheat-flour-mixture consisting of 21 flours out of 12 countries (Germany, France, Italy, Croatia, Austria, Czech Republic, UK, Russia, China, India, Thailand, USA) was used. The flours were common in commerce soft wheat flours with different refining grades. The unprocessed wheat-flour-mixture gave a recovery rate for gluten of about 134 % ± 25 % (n=15) in the spiking level sample of the PT DLA 03/2017 calculated from the ELISA method Ridascreen® Gliadin results.

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „blank"	**Level 1** 2 mg/kg	**Level 2** 10 mg/kg	**Level 3** 20 mg/kg	**Level 4** 50 mg/kg	**Level 5** 100 mg/kg
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Organic Maize-Chips, gluten free Ingredients: Maize flour (77%), sunflower oil, salt Nutrients per 100 g: Protein 6,7 g, carbo-hydrates 63 g, fat 22 g, salt 0,8 g	100	100	100	99,9	99,8	99,5
Maize-Chips (baked 195°C, 15 min) Ingredients: Maize flour, wheat-flour-mixture(25% in dry matter), water	–	0,0100	0,0500	0,100	0,249	0,498
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof Wheat: - Wheat flour* - *with 10% protein***	–	25,1 2,54	126 12,7	251 25,4	623 62,3	1245 126
- *thereof **Gluten****	–	**2,21**	**11,0**	**22,1**	**54,5**	**109**
Extended combined uncertainty (k=2) of Gluten-content (= ± 12 %)		± 0,26	± 1,3	± 2,6	± 6,5	± 13

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material: 10,1 ± 0,17% (total nitrogen according to Kjeldahl with F=5,7 for wheat protein)
*** Protein contents according to literature values (approx. 8,7% gluten in wheat flour [39, 40, 41])

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

Each assigned value, here the spiked allergen-contents, is afflicted with a standard uncertainty. As uncertainties the following factors were considered: protein content of spiking material, gluten content in soft wheat types, mixing homogeneity, homogeneity and stability of gluten.
All uncertainties were expressed in the form of their standard deviations and then added as variances. The square root from the sum of the total variances results in the combined uncertainty "Uc". Multiplied with the coverage factor k=2 the extended uncertainties of the assigned values "$U(X_{pt})$" are obtained [3, 13, 16-18].

2.1.1 Characterization of the PT-Sample series

The PT-sample series was characterized by ELISA (Immunolab Gliadin/Gluten ELISA, n=3). All 5 spiking levels were detected with a good correlation between spiking and mean of results (see Fig. 1). The relative standard deviation (RSD) was in the range of approx. 30% to 4,4% and the recovery rates ranged from 76% to 86%.

Table 2: Characterization of PT-sample series Gluten in Maize-Chips by ELISA determination (Immunolab Gliadin/Gluten, n=3).

PT-Sample	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
Spiking	0,0	2,2	11	22	55	109
Result 1	0,0	2,35	6,45	18,5	49,7	93,1
Result 2	0,0	1,58	9,17	19,9	43,2	92,2
Result 3	0,0	1,29	9,47	17,8	49,6	99,8
Mean	0,0	1,74	8,36	18,7	47,5	95,1
SD	-	0,55	1,66	1,08	3,71	4,16
RSD [%]	-	31,5	19,9	5,7	7,8	4,4
Recovery [%]	-	79,1	76,0	85,1	86,4	86,4

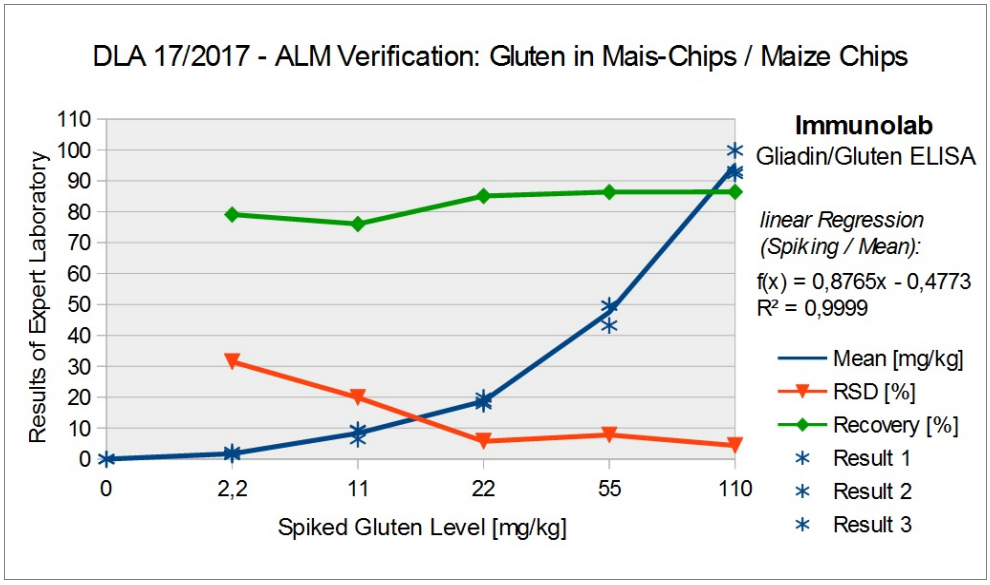


Abb./Fig. 1: ELISA results of PT-sample series Gluten in Maize-Chips (Immonuloab Gliadin/Gluten, n=3), *Note: the x-scale is not shown linear to obtain a better recognizability of low values.*

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples Level 1 to 5 showed a probability of 40%, 94%, 97%, 90% and 91%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat value of 1,3, 0,68, 0,68, 0,75 and 1,0 respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameter gluten for comparable food matrices and water activity (a_W value <0,5). The stability of the sample material is therefore given during the investigation period under consideration of given storage conditions.

2.2 Sample shipment and information to the test

The portion of test material (sample 1 to 6) were sent to every participating laboratory in the 13th week of 2017. The testing method was optional. The tests should be finished at May 12th 2017 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

The proficiency test Action Level Matrix (ALM) – Verification consists of five different samples with specified contents of gluten from wheat flour as well as a „blank sample" in the matrix maize chips.

- *The 6 samples are numbered in a random order.*
- *It is to be proven qualitatively by any suitable method that the so-called „Action Level" of 20 mg/kg gluten can be detected in the processed matrix (= Action Level 1 (VITAL concept 2.0) and judgement value of the German Commission ALTS/ALS).*
- *If possible, the indication of quantitative results is desirable in order to compare them with the levels of addition.*

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

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. total food item or protein in mg/kg were evaluated.
During evaluation DLA eventually requests detailed information by email on the type of indicated quantitative results from participants concerned.
Queried and documented were the indicated results and details of the test methods like specificity, 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 10 participants submitted results. Two of the participants submitted their results delayed.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are using different antibodies, which are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the analyte content [26-29, 40]. Furthermore matrix- and/or processing of samples can have a strong impact on the detectability of allergens by ELISA and/or PCR methods.

In the present PT the allergenic ingredient was provided in an especially processed food matrix in a kind of a calibration line with concentrations in the range of the so called Action Level. The allergen content here referred to as the "Action Level" is highlighted by colour in Table 3.

The participant results were evaluated qualitatively with an Action Level Matrix Score (*ALM-Score*), which indicates the number of successfully detected concentration levels.
The quantitative results were evaluated with a Recovery-Score (*RR-Score*), which indicates the number of results with a recovery rate in the range of 50 - 150% of the spiking level.

Table 3: Threshold doses, judgement values and legislative maximum values.(Highlighted by colour: Action Level in the present PT)[27, 42-44]

Allergen	Threshold dose * (Vital Concept 2.0)	Judgement value ALTS/ALS	Legislative Maximum value for declaration
	mg/kg	mg/kg	mg/kg
Gluten	***100***	***> 80***	***20 *****
Egg (as whole egg powder)	0,66	> 1	
Peanut	8	> 5	
Soy (as Soy flour)	25	> 20	
Milk (as defatted milk powder)	2,8	> 2,5	
Hazelnut	6,4	> 5	
Cashew	106	> 50	
Almond, Walnut, Pecan, Brazil-Nut, Pistachio, Macadamia	-	> 20	
Sesame, unpeeled	11,8	> 10	
Lupine	100	> 50	
Celery seed	-	> 20	
Mustard seed	1,9	> 5	

* calculated by threshold dose considering an intake of 100 g food [42, 43, 44]
** Maximum value for declaration as „gluten free" according to EU-VO 828/2014 [39]

3.1 Action Level Matrix Score (ALM-Score)

The qualitative valuation of each participant's results was performed with the so called ALM-Scores from 1-5 considering the number of "positive" or "negative" results matching the spiking of the PT-sample series (see Tab. 4). An ALM-Score from > 3 indicates a successful detection of the Action Level. The results of the matrix sample Level 0 were not evaluated if the participant result is in accordance with ≥75% positive or negative results of participants (consensus value) or if the result is below the limit of quantification of the used method.

Table 4: Evaluation of results using ALM-Scores

Level 0 „blank“	Level 1 2 mg/kg	Level 2 10 mg/kg	Level 3 (Action Level) 20 mg/kg	Level 4 50 mg/kg	Level 5 100 mg/kg	ALM-Score qualitative	Detection Action Level
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Levels 1 - 5	
negative	negative	negative	negative	negative	positive	1 (20%)	not successful
negative	negative	negative	negative	positive	positive	2 (40%)	not successful
negative	negative	negative	positive	positive	positive	3 (60%)	successful
negative	negative	positive	positive	positive	positive	4 (80%)	successful
negative	positive	positive	positive	positive	positive	5 (100%)	successful

3.2 Recovery-Score (RR-Score)

The evaluation of the quantitative participant results for the spiked **PT-samples** was done by recovery scores (*RR-Scores*) which are related to the number of recovery rates in the range of acceptance. The RR-Scores are calculated by counting the number of results in the range of acceptance (s. below) per number of quantitatively determined samples. Further the percentage is given in the brackets behind.
The recovery rates were calculated considering the content of spiked allergen (level of addition). The reference values are calculated from the values for Level 1 to 5 given in section 2.1 Sample material, Table 1. As range of acceptance RA for the evaluation of the participant results the range of the AOAC-recommendation of 50-150% for allergen-ELISAs was used [21]. This range was also used in the present PT for quantitative PCR-results.
Only exact quantitative results were considered. Single results outside the given measuring range (e.g. indicated with > 25 mg/kg or < 2,5 mg/kg) or indicated with "0" were not considered.

The given recovery rates enable inter alia an assessment of matrix and/or processing influences.

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 11% - 145% for PCR methods, depending on matrix or processing and concentration (s. Table 5a and 5b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 5a: ELISA-Methods – Recovery rates and precision data from chosen precision experiments[31-32].

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD_r	RSD_r	RSD_R	σpt	Method / Literature
Peanut	Milk chocolate	173,7 33,8 5,9	87 % 85 % 59 %	- - -	8,8% 5,2% 7,8%	31% 20% 31%	30,4% 19,7% 30,5%	ELISA Manuf. A ASU 00.00-69
Peanut	Milk chocolate	215,7 40,1 10,1	108 % 100 % 101 %	- - -	5,9% 7,2% 7,3%	32% 14% 16%	31,7% 13,0% 15,1%	ELISA Manuf. B ASU 00.00-69
Peanut	Dark chocolate	148,2 30,9 5,7	74 % 77 % 57 %	- - -	6,0% 13% 6,1%	22% 25% 33%	21,6% 23,2% 32,7%	ELISA Manuf. A ASU 00.00-69
Hazelnut	Dark chocolate	16,3 7,56 3,73 1,62	81 % 76 % 75 % 81 %	- - - -	4,7% 8,9% 13% 15%	12% 15% 24% 33%	11,5% 13,6% 22,2% 31,2%	ELISA Manuf. A ASU 44.00-7
Hazelnut	Dark chocolate	21,3 10,7 4,69 2,37	106 % 107 % 94 % 119 %	- - - -	7,1% 11% 11% 9,3%	14% 19% 17% 17%	13,1% 17,3% 15,1% 16,4%	ELISA Manuf. B ASU 44.00-7

The Working Group on Prolamin Analysis and Toxicity (WGPAT)performed ring trials for validation of two commercial ELISA-Kits for determination of gluten using monoclonal R5 antibodies [25]. 12 food samples with gliadin contents in the range if 0 - 168 mg/kg were analysed by 20 laboratories. The obtained recovery rates were in the range between 65 and 110%, the relative repeatability standard deviation was between 1 – 25% (1. method) and 11 - 22% (2. method) and the relative reproducibility standard deviation between 23 - 47 % (1. method) and 25 - 33% (2. method). The authors concludes that both ELISA-Kits fulfil the validation criteria for ELISA methods.

THE IRMM (Institute for Reference Materials and Measurements) proofed the suitability of five different ELISA-Kits for the determination of peanut [28]. The mean values were in the concentration range of 0,3 - 16,1 mg/kg and/or 1,2 - 20,4 mg/kg. The smallest relative reproducibility standard deviation for each Kit was obtained for dark chocolate at 20 - 42% and cookies at 23 - 61%.

Table 5b: PCR-Methods - Relative repeated standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) according to chosen evaluation from experiments by precision and the resulting target standard deviation σ_{pt} [33-38].

Parameter	**Matrix**	**Mean [mg/kg]**	**Reco-very**	**rob RSD_r**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
Almond	Rice cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR ASU 18.00-22
Sesame	Rice cookie	94,6 15,7 9,8	95 % 79 % 98 %	-	22,5% 26,0% 20,9%	27,5% 39,5% 33,5%	22,4% 35,0% 30,0%	rt-PCR ASU 18.00-19
Sesame	Wheat cookie Sauce powder	96,9 59,8	79 % 60 %	-	21,8% 22,2%	33,0% 43,2%	29,2% 40,2%	rt-PCR ASU 18.00-19
Sesame	Rice cookie	88,9 17,8 9,8	89 % 89 % 98 %	-	18,2% 34,2% 26,2%	30,5% 37,8% 37,0%	27,7% 29,1% 32,0%	rt-PCR ASU 18.00-22
Sesame	Wheat cookie Sauce powder	115 58,5	93 % 59 %	-	16,7% 30,8%	41,1% 44,4%	39,4% 38,7%	rt-PCR ASU 18.00-22
Soy	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Wheat + Rye	Boiled saus-age (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.2.1 Recovery rates by precision experiment

Requirements to the performance of analysis methods for quantitative determination of allergens in food were compiled for example from the Ministry of Health and Welfare (MHLW) in Japan [23], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [20-22], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [24] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [19].

The following relevant ELISA and/or PCR validation criteria of the committees are given in Table 6 and 7.

Table 6: ELISA validation criteria

Literature [19-25]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% (a)	19,5 - 57,2% (a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

(a) = Example from hypothetical ring trail in the concentration range of 0,5 - 5 mg/kg

Table 7: PCR validation criteria

Literature [19]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Due to the current performance of ELISA and PCR methods for quantitative determination of allergens in food, which can be derived from precision data by experiments and from validation criteria mentioned above, a common relative target standard deviation (σ_{pt} value) from 25% was defined. The recovery rate was set to 50-150%.

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done for ELISA and PCR-techniques together. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

In the result chapter all quantitative results of the participants are displayed formatted to 3 decimal places. In the documentation, all results are given as they were transmitted by the participants.

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

In the present PT all results were given as gluten, therefore no recalculation was necessary.

The qualitative results are presented in the corresponding evaluation table as indicated below:

Participant	Level 0 „blank“	Level 1 2 mg/kg	Level 2 10 mg/kg	Level 3 (Action Level) 20 mg/kg	Level 4 50 mg/kg	Level 5 100 mg/kg	ALM-Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Levels 1 - 5		

In cases when quantitative values were submitted the result table are given as indicated below:

Participant	Level 1 - 2 mg/kg		Level 2 - 10 mg/kg		Level 3 - 20 mg/kg (Action Level)		Level 4 - 50 mg/kg		Level 5 - 100 mg/kg		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		

* RR = Recovery Rate (RR)

4.1 Proficiency Test Gluten

4.1.1 Qualitative: Action Level Matrix – Scores (ALM-Scores)

Evaluation number	Level 0 „blank“	Level 1 2 mg/kg	Level 2 10 mg/kg	Level 3 (Action Level) 20 mg/kg	Level 4 50 mg/kg	Level 5 100 mg/kg	ALM-Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Levels 1 - 5		
2a	negative	positive	positive	positive	positive	positive	5 (100%)	AQ	
5	negative	positive	positive	positive	positive	positive	5 (100%)	IL	Mean of 3 determinations
1	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
2b	negative	positive	positive	positive	positive	positive	5 (100%)	RS	
3	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
4	positive	positive	positive	positive	positive	positive	5 (100%)	RS	Level 0 and 1: < 5 mg/kg
6	negative	positive	positive	positive	positive	positive	5 (100%)	RS	
7	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
8	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	
9	positive	positive	positive	positive	positive	positive	5 (100%)	VT	blank sample: 0,48 mg/kg
10a	negative	positive	positive	positive	positive	positive	5 (100%)	VT	
10b	negative	positive	positive	positive	positive	positive	5 (100%)	div	PCR-Method

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	2	9	12	12	12	12
Number negative	10	3	0	0	0	0
Percent positive	17	75	100	100	100	100
Percent negative	83	25	0	0	0	0
Consensus value	negative	positive	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

AQ = AgraQuant, RomerLabs

IL = Immunolab

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox R5, Neogen

div = not indicated / other method

Comments:

All participants detected successfully level 2 and thus the half of the gluten content of the Action Level in the processed matrix Maize-Chips. The lowest content of 2 mg/kg (1/10 of the Action Level) was still detected by 75% of the participants. This value is in the range or below the limit of quantification of used methods.

4.2.1 Quantitative: Recovery-Scores (RR-Scores)

Evaluation number	Level 1 - 2 mg/kg		Level 2 - 10 mg/kg		Level 3 - 20 mg/kg (Action Level)		Level 4 - 50 mg/kg		Level 5 - 100 mg/kg		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA **		
2a	4,80	217	10,0	**91**	38,0	172	120	220	240	220	1/5 (20%)	AQ	
5	1,74	**79**	8,36	**76**	18,7	**85**	47,5	**87**	95,1	**87**	5/5 (100%)	IL	Mean of 3 determinations
1	<5		15,0	**136**	23,0	**104**	70,0	**128**	160	**147**	4/4 (100%)	RS	
2b	4,40	199	17,0	155	30,0	**136**	78,0	**143**	160	**147**	3/5 (60%)	RS	
3	< 5		15,9	**145**	28,2	**128**	98,1	180	151	**139**	3/5 (60%)	RS	
4	< 5		16,5	**150**	40,3	182	70,5	**129**	179	164	2/4 (50%)	RS	
6	3,76	170	14,5	**132**	29,4	**133**	89,3	164	180	165	2/5 (40%)	RS	
7	<3		17,0	155	37,0	167	118	217	268	246	0/4 (0%)	RS	
8	5,30	240	12,5	**114**	33,0	**149**	61,0	**112**	199	183	3/5 (60%)	RS-F	
9	6,09	276	13,7	**125**	25,9	**117**	57,6	**106**	108	**99**	4/5 (80%)	VT	
10a	<5		22,0	200	40,0	181	90,0	165	196	180	0/4 (0%)	VT	
10b	2,80	**127**	15,0	**136**	23,0	**104**	54,0	**99**	100	**92**	5/5 (100%)	div	PCR-method
	RA**	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**			
	Anzahl im AB	**2**	**Anzahl im AB**	**9**	**Anzahl im AB**	**8**	**Anzahl im AB**	**7**	**Anzahl im AB**	**6**			
	Prozent im AB	**29**	**Prozent im AB**	**75**	**Prozent im AB**	**67**	**Prozent im AB**	**58**	**Prozent im AB**	**50**			

* Recovery Rate 100% refernce value: Gluten, s. Page 6

** Range of acceptance by AOAC for Allergen-ELISAs

Methods:

AQ = AgraQuant, RomerLabs

IL = Immunolab

RS = Ridascreen®, R-Biopharm

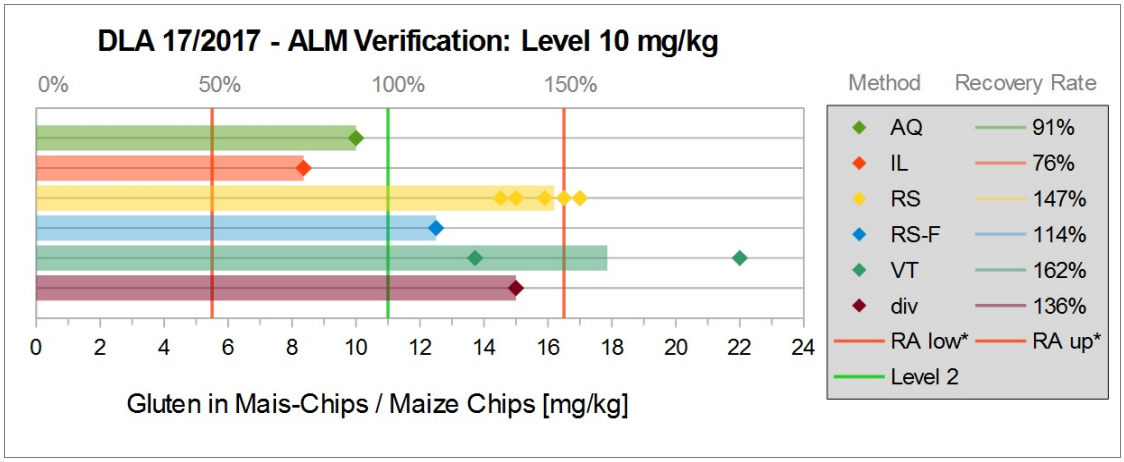
RS-F= Ridascreen® Fast, R-Biopharm

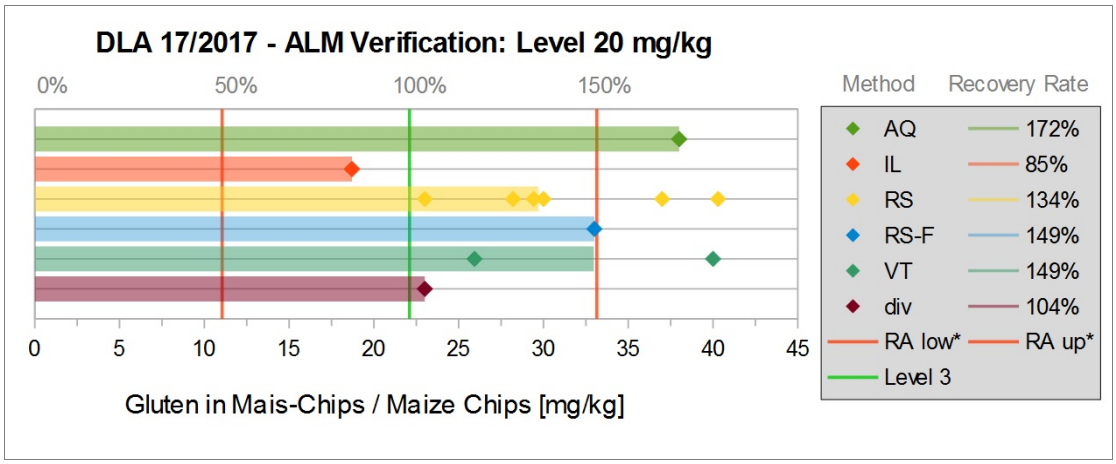
VT = Veratox R5, Neogen

div = not indicated / other method

Comments:

For levels 2 to 5 there were 50-75% of the recovery rates of the participant results within the range of acceptance of 50-150%.





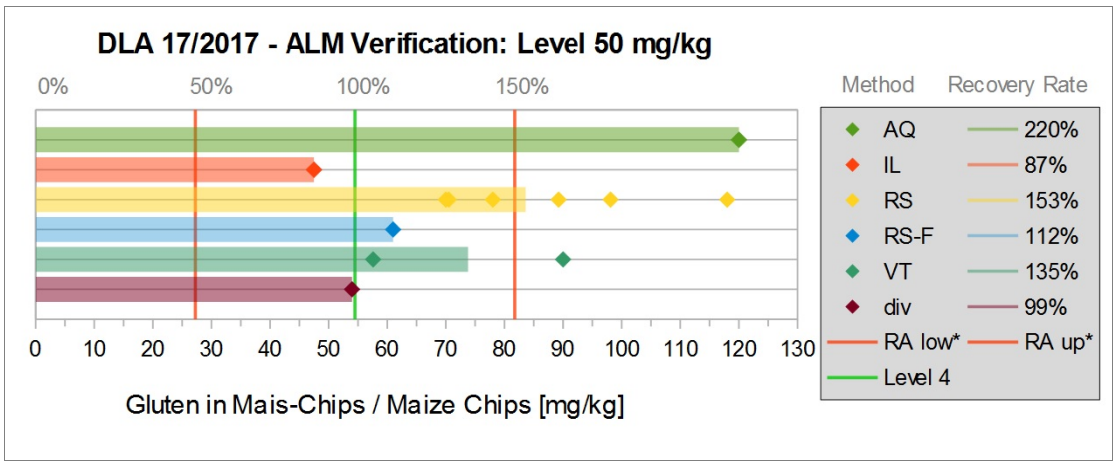


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale gluten content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 Gluten

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample 6 Blank Sample		Result Sample 1 2 mg/kg Level		Result Sample 4 10 mg/kg Level		Result Sample 2 20 mg/kg Level		Result Sample 5 50 mg/kg Level		Result Sample 3 100 mg/kg Level		NWG / LOD *	BG / LOQ *	Result given as
		Day/Month	qualtitative	mg/kg	qualtitative	mg/kg	qualtitative	mg/kg	qualtitative	mg/kg	qualtitative	mg/kg	qualtitative	mg/kg	mg/kg	mg/kg	
AQ	2a	03.05.17	negative	< 4	positive	4,8	positive	10	positive	38	positive	120	positive	240	2	4	Gluten
IL	5	04.03.17	negative	0	positive	1,74	positive	8,36	positive	18,7	positive	47,5	positive	95,1	0,6	4	Gluten
RS	1	13.4.	negative	<5	negative	<5	positive	15	positive	23	positive	70	positive	160	3	5	Gluten
RS	2b	03.05.17	negative	< 5	positive	4,4	positive	17	positive	30	positive	78	positive	160	1	5	Gluten
RS	3	27./28.04.	negative	< 5	negative	< 5	positive	15,9	positive	28,2	positive	98,1	positive	151,4	5	5	Gluten
RS	4	20.04.17	positive	< 5	positive	< 5	positive	16,5	positive	40,3	positive	70,5	positive	179,3	1	5	Gluten
RS	6a	19.04.17	negative		positive		positive		positive		positive		positive		1	5	Gluten
RS	6b	19.04.17	-	< LOD	-	3,76	-	14,52	-	29,42	-	89,25	-	179,75	1	5	Gluten
RS	7	11.05.17	negative	<3	negative	<3	positive	17	positive	37	positive	118	positive	268	3	5	Gluten
RS-F	8	10.05.17	negative		positive	5,3	positive	12,5	positive	33	positive	61	positive	199	3	5	Gluten
VT	9	19.04.	-	0,48	-	6,09	-	13,72	-	25,93	-	57,58	-	107,71			Gluten
VT	10a	19.4./15.5.17	negative	<2	positv	<5	positive	22	positive	40	positive	90	positive	196	2	5	Gluten
div	10b	10.05.17	negative	<2	positive	2,8	positive	15	positive	23	positive	54	positive	100	2	2	Gluten

Continuation details by participants

Meth. Abr.	Evaluation number	Method	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	e.g. Extraction solution / Time / Temperature	yes/no	
AQ	2a	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs		As per kit instructions	no	
IL	5	Immunolab Gliadin/Gluten ELISA	Polyclonal	Each sample was extracted 3-fold and the extracts were determined in double dtermination with an actual charge (GLI-143) of Immunolab Gliadin ELISA. The results are already given in Gluten. The raw data (c=Gliadin) are documented beside.		Mean out of 3 determinations
RS	1	Ridascreen® Gliadin R7001, R-Biopharm	R5 Mendez, recognises Prolamine from wheat/ rye/ barley	As per kit instructions (cocktail treatment)	yes	Limit of detection given by kit provider (1 ppm) can not be confirmed, thus 3 ppm were indicated as limit of detection; Result Sample 1: <5ppm, traces in the range of detection limit were recognised; Result Sample Probe 6: <5ppm, <3ppm
RS	2b	Ridascreen® Gliadin R7001, R-Biopharm		As per kit instructions with cocktail-solution R7016	yes	Standard 2 (5ppb) 2x 1:2 diluted, to determine Concentration 2 mg/kg
RS	3	Ridascreen® Gliadin R7001, R-Biopharm	R5	As per kit instructions	yes	
RS	4	Ridascreen® Gliadin R7001, R-Biopharm	R5	Cocktail (R7006/R7016) according to kit instruction	no	
RS	6a	Ridascreen® Gliadin R7001, R-Biopharm	R5-antibody	Cocktail patented, official R5-Mendez-Method	no	see separate E-Mail*
RS	6b	Ridascreen® Gliadin R7001, R-Biopharm	R5-antibody	Cocktail patented, official R5-Mendez-Method	no	see separate E-Mail*
RS	7	Ridascreen® Gliadin R7001, R-Biopharm			yes	
RS-F	8	Ridascreen® FAST Gliadin R7002, R-Biopharm	R5	As per kit instructions	yes	
VT	9	Veratox Gliadin R5, Neogen				
VT	10a	Veratox Gliadin R5, Neogen	Gliadin R5	According to Veratox Gliadin R5, Neogen	yes	
div	10b	internal PCR-Method	gamma-Gliadin from wheat	NucleoSpin Food (Macherey-Nagel) / Real Time PCR	qualitative determination	Analyt wheat-DNA

* the E-Mail contains questions towards sample material, the corresponding informtion is included in the present report

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 17-2017 Sample 2 mg/kg

Weight whole sample	0,74	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,17	75	29,0
2	5,10	75	29,4
3	5,03	54	21,5
4	5,16	69	26,7
5	5,10	66	25,9
6	5,12	52	20,3
7	5,07	63	24,9
8	5,06	64	25,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	64,7	Particles
Standard deviation	8,23	Particles
χ^2 (CHI-Quadrat)	7,32	
Probability	**40**	%
Recovery rate	87	%

Normal distribution		
Number of samples	8	
Mean	25,4	mg/kg
Standard deviation	3,23	mg/kg
rel. Standard deviaton	12,7	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,3**	
Recovery rate	87	%

Microtracer Homogeneity Test
DLA 17-2017 Sample 10 mg/kg

Weight whole sample	0,75	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	38,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,18	78	30,1
2	5,09	86	33,8
3	4,97	74	29,8
4	5,07	80	31,6
5	5,11	84	32,9
6	4,96	86	34,7
7	5,15	83	32,2
8	5,01	72	28,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	80,4	Particles
Standard deviation	5,23	Particles
χ^2 (CHI-Quadrat)	2,39	
Probability	**94**	%
Recovery rate	83	%

Normal distribution		
Number of samples	8	
Mean	31,7	mg/kg
Standard deviation	2,07	mg/kg
rel. Standard deviaton	6,51	%
Horwitz standard deviation	9,51	%
HorRat-value	**0,68**	
Recovery rate	83	%

Microtracer Homogeneity Test
DLA 17-2017 Sample 20 mg/kg

Weight whole sample	1,25	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	27,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	58	23,1
2	5,08	61	24,0
3	5,05	53	21,0
4	5,00	50	20,0
5	5,16	51	19,8
6	5,03	54	21,5
7	5,00	52	20,8
8	5,09	53	20,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,0	Particles
Standard deviation	3,73	Particles
χ^2 (CHI-Quadrat)	1,80	
Probability	**97**	%
Recovery rate	78	%

Normal distribution		
Number of samples	8	
Mean	21,4	mg/kg
Standard deviation	1,48	mg/kg
rel. Standard deviaton	6,90	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,68**	
Recovery rate	78	%

Microtracer Homogeneity Test
DLA 17-2017 Sample 50 mg/kg

Weight whole sample	0,75	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	36,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	80	31,7
2	4,96	86	34,7
3	5,02	76	30,3
4	5,15	74	28,7
5	4,96	84	33,9
6	5,11	76	29,7
7	5,01	78	31,1
8	5,09	73	28,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	78,4	Particles
Standard deviation	5,62	Particles
χ^2 (CHI-Quadrat)	2,82	
Probability	**90**	%
Recovery rate	85	%

Normal distribution		
Number of samples	8	
Mean	31,1	mg/kg
Standard deviation	2,23	mg/kg
rel. Standard deviaton	7,16	%
Horwitz standard deviation	9,54	%
HorRat-value	**0,75**	
Recovery rate	85	%

Microtracer Homogeneity Test
DLA 17-2017 Probe 100 mg/kg

Weight whole sample	1,15	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	29	11,6
2	5,05	34	13,5
3	4,97	30	12,1
4	5,11	28	11,0
5	5,03	35	13,9
6	4,97	38	15,3
7	5,09	31	12,2
8	5,07	34	13,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	32,4	Particles
Standard deviation	3,58	Particles
χ^2 (CHI-Quadrat)	2,76	
Probability	**91**	%
Recovery rate	55	%

Normal distribution		
Number of samples	8	
Mean	12,9	mg/kg
Standard deviation	1,42	mg/kg
rel. Standard deviaton	11,0	%
Horwitz standard deviation	10,9	%
HorRat-value	**1,0**	
Recovery rate	55	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***DLA 17-2017***
PT name	***ALM-Verification Gluten: 5 samples containing Wheat Flour in Maize-Chips-Matrix*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Maize-Chips (gluten containing samples: 195°C, 15 min) / ingredients: maize flour, sunflower oil, salt and allergenic food wheat flour (only in gluten containing samples)*
Number of samples and sample amount	*5 different Samples: 20 g each + 1 „blank sample" : 20 g*
Storage	*Samples : room temperature (long term 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative (optional: quantitative) Gluten / gluten containing cereals Levels: 0, 2, 10, 20, 50 and 100 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably the total sample amount should be homogenized.*
Result sheet	*One qualitative (and optional quantitative) result each should be determined for Samples 1-6. The results should be filled in the result submission file.*
Units	*positive / negative (optional: mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest May 12th 2017***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		SWITZERLAND
		Germany
		ITALY
		AUSTRIA
		Germany
		Germany
		Germany
		Germany
		Germany

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

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

7. Index of references

1. 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
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. 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)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. EN ISO/IEC 17034:2016; Konformitätsbewertung - Allgemeine Anforderungen an die Kompetenz von Referenzmaterialherstellern / General requirements for the competence of reference material producers
17. ISO Guide 34:2000; General requirements for the competence of reference material producers
18. DAkkS 71 SD 1/4 016; Ermittlung und Angabe der Messunsicherheit nach Forderungen der DIN EN ISO/IEC 17025 (2011) [Estimation and indication of the measurement uncertainty]
19. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, CAC/GL 74-2010
20. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General

considerations
21. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
22. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
23. Ministry of Health and Welfare, JSM, Japan 2006
24. 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)
25. 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)
26. 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)
27. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
28. 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
29. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
30. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007)
31. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003)
32. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006)
33. ASU §64 LFGB L 16.01-9 Untersuchung von Lebenmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016)
34. ASU §64 LFGB L 18.00-19 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Sesam (Sesamum indicum) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014)
35. ASU §64 LFGB L 18.00-20 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014)
36. ASU §64 LFGB L 18.00-22 Untersuchung von Lebenmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014)
37. ASU §64 LFGB L 08.00-59 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013)
38. ASU §64 LFGB L 08.00-66 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Weizen (Triticum L.) und Roggen (Secale cereale) in Brühwurst mittels real-time PCR (2016)
39. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Information-en für Verbraucher über das Nichtvorhandensein oder das reduzierte Vorhandensein von Gluten in Lebensmitteln / on the requirements for the provision of information to consumers on the absence or reduced presence

of gluten in food
40. Bruins-Slot et al. (2015) Evaluating the performance of gluten ELISA test kits: The numbers do not tell the tale, Cereal Chem 92(5):513-521
41. Köhler & Andersen (2014) Analyse von Glutengehalten in Getreide und getreidehaltigen Produkten, Tabellenwerk zum Nährstoffgehalt von Lebensmitteln 3.1.5.1, Deutsche Forschungsanstalt für Lebensmittelchemie Leibniz Institut Jahresbericht 2014 [Analysis of gluten contents in cereals and cereal products, nutrient tables of foods]
42. Allen et al. (2014) Allergen reference doses for precautionary labeling (VITAL 2.0): clinical implications, J Allergy Clin Immunol 133:156-164
43. Taylor et al. (2014) Establishment of reference doses for residues of allergenic foods: report of the VITAL Expert Panel, Food Chem Toxicol 63: 9-17
44. Demmel et al. (2015) Kap. 4.1 Existierende Aktionswerte, in: Allergene in Lebensmitteln, Behr's Verlag, Hamburg [Chapter 4.1 Existing Action Levels, in Allergens in Foods]