Evaluation Report
proficiency test

DLA ptALR2 (2021)

Response PT Gluten:

5 processed Samples Wheat Flour, Bread, Noodles (Durum Wheat), Bulgur (pre-cooked Wheat) and Seitan (Gluten Protein)

in Potato Powder Matrix

DLA - Proficiency Tests GmbH
Hauptstr. 80
23845 Oering/Germany

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

Coordinator of this PT:
Matthias Besler-Scharf, PhD.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Hauptstr. 80, 23845 Oering, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptALR2 (2021)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (16 February 2022) 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-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 16 February 2022
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficency test the following services were subcontracted: protein determination
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.

Contents

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 proficiency test format „**Response PT Allergens**" includes 5 differently processed samples of an allergen in a simple carrier matrix as well as a "blank sample". Hereby it offers the possibility to prove that the analytical determination methods used by the participants are suitable to detect the respective processed allergens qualitatively and to determine its quantitative response factors.

In order to ensure comparability of the processed sample material, the allergen contents of the PT sample series were adjusted to approximately the same levels calculated as gluten contents. The evaluation of the PT-results was done qualitatively by scores from 1-5 (score 5 = all processings successfully determined). Quantitative results were given including the calculated respective recovery rate (recovery score) for information in the report.

2. Realisation

2.1 Test material

6 PT-samples for qualitative and optionally quantitative determination of gluten in wheat flour, bread, noodles (durum wheat), bulgur (pre-cooked durum wheat) and seitan (gluten protein) in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce processed wheat products. For each PT-sample 5-21 products of different origin were worked up. After cooking, the seitan preparation was freeze-dried before further use.

Afterwards premixes with contents from approx. 6 - 10 % of the regarding allergenic ingredients as gluten were produced (s. Tab. 1). For this the products were pre crushed if necessary, mixed gravimetrically with further ingredients, crushed and sieved by means of a centrifugal mill (mesh 250 µm) and homogenized. The allergen-premixes were added to the carrier matrix of potato powder / maltodextrin (mesh < 500 µm) and homogenized. An aliquot of the carrier matrix was provided as the "blank sample".

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

The gluten contents of the PT-samples were approx. 30-44 mg/kg (see Tab. 1).

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 materials, 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, 16-17].

Table 1: Composition of DLA-Samples

PT-Sample series	**Sample 1** **Noodles**	**Sample 2** **Bread**	**Sample 3** **Bulgur**	**Sample 4** **Wheat Flour**	**Sample 5** **Seitan**	**Sample 6** **„blank"**
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Potato powder Ingredients: potato, E471, E304, E223, E100 Nutrients per 100 g: Protein 8,3 g, carbohydrates 76 g, fat 0,6 g, salt 0,15 g	75	75	75	75	75	75
Maltodextrin	25	25	25	25	25	25
Allergen-Premixes Ingredients: processed allergen products (each 6% - 10% gluten) and maltodextrin (seitan only)	0,042	0,046	0,040	0,041	0,045	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Noodles (Durum wheat)* Ingredients: Durum wheat semolina, water Protein 12,4% ** *(5 Dry products from Europe)*	418	-	-	-	-	-
White Bread (Soft wheat)* Ingredients: wheat flour, water, yeast, salt, rapeseed oil, acidity regulator E262, invert sugar syrup, wheat malt flour, antioxidant E300 Protein 11,1% ** *(5 Products from Germany)*	-	456	-	-	-	-
Bulgur (Durum wheat)* Ingredients: Bulgur (Durum wheat, pre-cooked) Protein 11,8% ** *(5 Dry products from Europe)*	-	-	402	-	-	-
Wheat flour (Soft wheat)* Protein 10,1 % ** *(21 Products from Europe, Asia, USA)*	-	-	-	414	-	-
Seitan (Wheat protein) (boiled 100°C, 30 min, freeze-dried)* Ingredients: Seitan powder (#), water, salt Protein 69,9% ** *(# 5 Products from Europe)*	-	-	-	-	44,8	-
- thereof Gluten	33,0	43,5	30,1	36,0	29,8	-
Extended combined uncertainty (k=2) of gluten content (= ± 12 %)	± 3,96	± 5,22	± 3,61	± 4,32	± 3,58	-

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture

** Protein contents according to laboratory analysis of raw materials (total nitrogen according to Kjeldahl with F=5,7 for wheat protein)

*** Gluten contents calculated according to literature values (for soft wheat approx. 8,7% gluten in soft wheat flours (by DLA related to 10,1% total protein) [39]; for durum wheat approx. 7,7% gluten and 12,1% total protein in durum wheat flours) [40]); for seitan approx. 79,9% gluten and 83,5% total protein in vital gluten products [41])

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

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 1 to 5 showed a probability of 44%, 47%, 72%, 79% and 78%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 1,2, 1,2, 0,95, 1,1 and 0,92 respectively. The results of the microtracer analysis are given in the documentation.

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,36 - 0,46 (20,5 - 21,4°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 39th week of 2021. The testing method was optional. The tests should be finished at November 26th 2021 the latest.

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

There are 5 different samples with similar contents of the allergenic parameter gluten which is differently processed, contained in a simple carrier matrix as well as a "blank"-sample (carrier matrix).

- *The samples 1-5 are numbered in a random order. They contain Wheat Flour, Bread, Noodles (Durum Wheat), Bulgur (pre-cooked Wheat) and Seitan (Gluten Protein)*
- *Please give all your quantitative results as gluten, or wheat / gluten-containing cereals.*
- *Possible conversion factors for processed gluten products are queried separately in the result submission file.*

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 in mg/kg were evaluated.
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.

12 out of 13 participants submitted results at least for one method. One participant submitted no results.

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 five different processed products containing the allergen gluten, wheat flour, bread, noodles (durum wheat), bulgur (pre-cooked durum wheat) and seitan (gluten protein), were provided to determine the qualitative detectability and to determine the response in the used quantitative methods.

The participant results were evaluated *qualitatively* with a score from 1-5 indicating the number of successfully detected processed products.
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.

3.1 Qualitative Score

The qualitative valuation of each participant's results was performed with Scores from 1-5 considering the number of "positive" or "negative" results matching the **spiking of the PT-sample series** (see Tab. 2).
A Score from 5 indicates, that all processed products were detected successfully.
The results of the matrix sample no. 6 ("blank"-sample) 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 2: Evaluation of results using qualitative Scores

Sample 1 Noodles	Sample 2 Bread	Sample 3 Bulgur	Sample 4 Wheat Flour	Sample 5 Seitan	Sample 6 „blank"	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not sucessful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

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 the spiked allergen (level of addition). The reference values are calculated from the values for samples 1 to 5 given in section 2.1 Sample material in 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- and LC/MS-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 – 120% for PCR methods (wheat and rye, gluten), depending on matrix or processing and concentration (s. Table 3a and 3b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 3a: 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 [31]. 12 food samples with gliadin contents in the range of 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 13 – 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 [31].

THE IRMM (Institute for Reference Materials and Measurements) proofed the suitability of five different ELISA-Kits for the determination of peanut [34]. 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 3b: 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} [32-36]

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD_r	RSD_r	RSD_R	σ_{pt}	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 sausage (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

3.2.2 Values by perception

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 [25], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [22-24], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [26] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [21].

The following relevant ELISA and/or PCR validation criteria of the committees are given in Table 4 and 5.

Table 4: ELISA validation criteria

Literature [21-26]	**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 5: PCR validation criteria

Literature [20]	**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%.

3.3 z-Score (Spiking Levels)

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}), here the spiking levels [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

The z-scores corresponding to the recovery rates were calculated with the target standard deviation of 25% (see 3.2.2).

3.4 z'-Score (Spiking Levels)

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered. The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(X_{pt})}$) [3].

The calculation is performed by:

$$z_i' = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.
The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

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 separately for ELISA- (and Lateral Flow) and PCR-methods.

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:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5		

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

Evaluation number	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		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**		

* Recovery Rate

4.1 Proficiency Test Processed Wheat Products

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Sample 1 Noodles	Sample 2 Bread	Sample 3 Bulgur	Sample 4 Wheat Flour	Sample 5 Seitan	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
11	positive	positive	positive	positive	positive	negative	5 (100%)	PB	
1	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
3	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
7	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
8	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
10	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
10	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
2a	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
4	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
9	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
2b	positive	positive	positive	positive	positive	negative	5 (100%)	SP-R5	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	12	12	12	12	12	0
Number negative	0	0	0	0	0	12
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

PB = Allergen-Shield ELISA, ProGnosis Biotech

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP-R5 = SensiSpec INgezim Gluten R5, Eurofins

Comments:

For all processed products (samples 1 to 5) consensus values of 100% positive results were obtained by the ELISA-methods.

4.1.2 Qualitative Scores: PCR-Methods

Evaluation number	Sample 1 Noodles	Sample 2 Bread	Sample 3 Bulgur	Sample 4 Wheat Flour	Sample 5 Seitan	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
5	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
10	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-Q	
2	positive	positive	positive	positive	positive	negative	5 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	3	3	3	3	3	0
Number negative	0	0	0	0	0	3
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
For all samples 1-5 consensus values of 100% positive results were obtained by PCR-methods.

4.1.3 Quantitative: ELISA-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Noodles			Sample 2 Bread			Sample 3 Bulgur			Sample 4 Wheat Flour			Sample 5 Seitan			RR- score	Method	Remarks
	Result	RR *		Result	RR *		Result	RR *		Result	RR *		Result	RR *		RR *		
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	Number in RA **		
11	34,2	**104**	0,15	80,0	184	3,4	20,2	**67**	-1,3	58,6	163	2,5	58,2	195	3,8	2/5 (40%)	PB	
1	17,8	**54**	-1,8	58,1	**134**	1,3	12,3	41	-2,4	51,9	**144**	1,8	43,2	**145**	1,8	4/5 (80%)	RS	
3																	RS	
7	21,5	**65**	-1,4	58,6	**135**	1,4	16,9	**56**	-1,8	32,8	**91**	-0,35	32,8	**110**	0,40	5/5 (100%)	RS	
8	19,7	**60**	-1,6	52,0	**120**	0,78	14,8	49	-2,0	44,8	**124**	0,98	36,8	**123**	0,94	5/5 (100%)	RS	
10	18,1	**55**	-1,8	63,3	**146**	1,8	19,2	**64**	-1,4	43,6	**121**	0,84	34,3	**115**	0,60	5/5 (100%)	RS	
12	25,8	**78**	-0,87	66,6	153	2,1	13,0	43	-2,3	35,6	**99**	-0,04	42,8	**144**	1,7	3/5 (60%)	RS	
2a	34,0	**103**	0,12	61,0	**140**	1,6	23,0	**76**	-0,94	55,0	153	2,1	49,0	164	2,6	3/5 (60%)	RS-F	
4	20,9	**63**	-1,5	61,0	**140**	1,6	17,9	**59**	-1,6	47,9	**133**	1,3	33,3	**112**	0,47	5/5 (100%)	RS-F	
6	34,0	**103**	0,12	62,0	**143**	1,7	18,0	**60**	-1,6	56,0	156	2,2	60,0	201	4,1	3/5 (60%)	RS-F	
9	20,7	**63**	-1,5	45,5	**105**	0,19	15,3	**51**	-2,0	36,8	**102**	0,09	36,7	**123**	0,93	5/5 (100%)	RS-F	
2b	33,0	**100**	0,00	41,0	**94**	-0,23	9,20	31	-2,8	44,0	**122**	0,89	29,0	**97**	-0,11	4/5 (80%)	SP-R5	
	RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %				
	Number in RA	11		Number in RA	9		Number in RA	8		Number in RA	8		Number in RA	8				
	Percent in RA	100		Percent in RA	82		Percent in RA	73		Percent in RA	73		Percent in RA	73				

*Recovery rate 100% Reference value: Gluten, see page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:
PB = Allergen-Shield ELISA, ProGnosis Biotech
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP-R5 = SensiSpec INgezim Gluten R5, Eurofins

Comments:
For samples 1-5, 73-100% of the recovery rates of the participant results were within the 50-150% acceptance range. The mean recovery rates for the soft wheat products were above 100% (bread 136%, wheat flour 128% and seitan 139%). In contrast, the recovery rates for products made from durum wheat were below 100% at 77% (pasta) and 54% (bulgur).

4.1.4 Quantitative: PCR-Methods Recovery Rates-Scores (RR-Scores)

No quantitative results were submitted for the PCR methods.

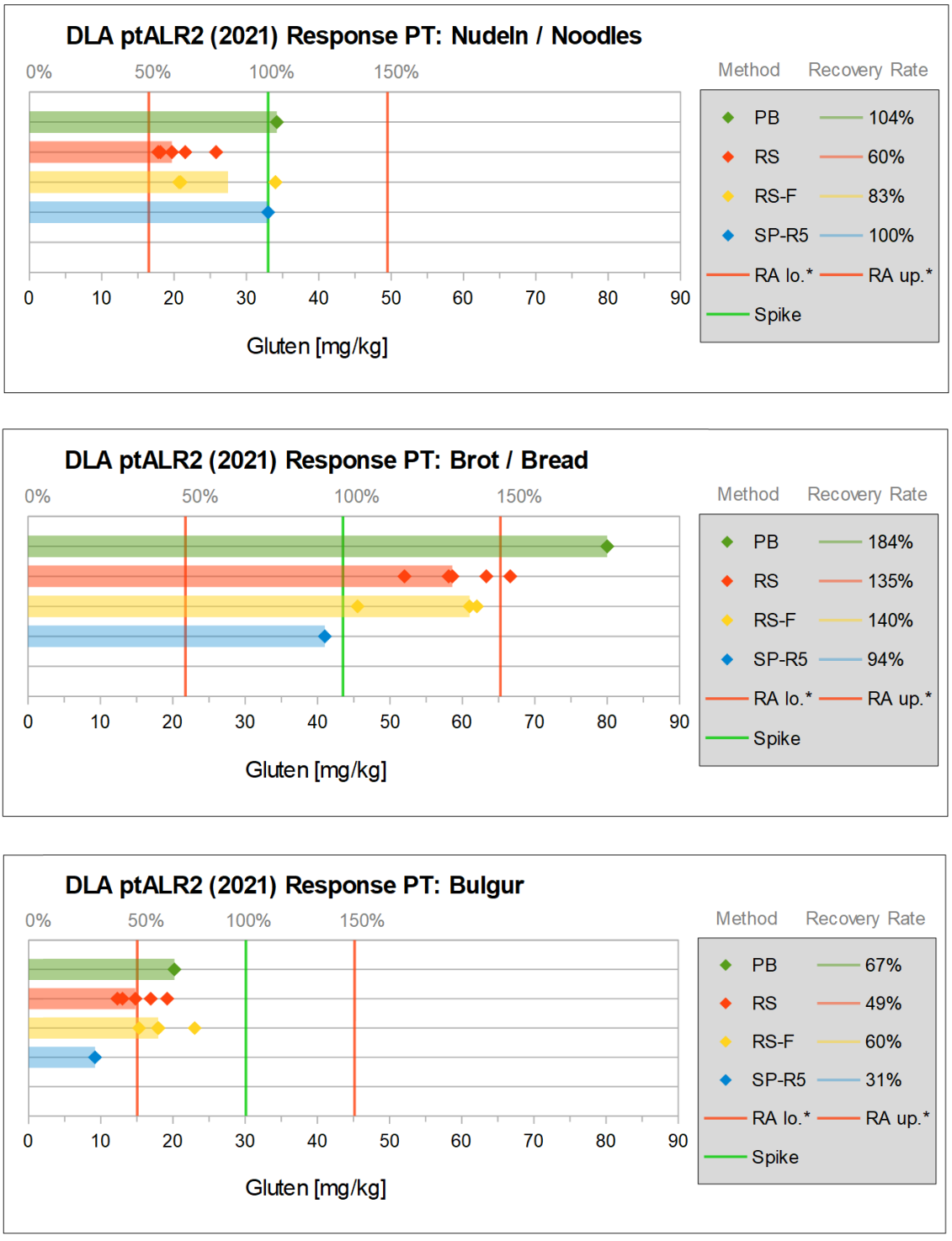
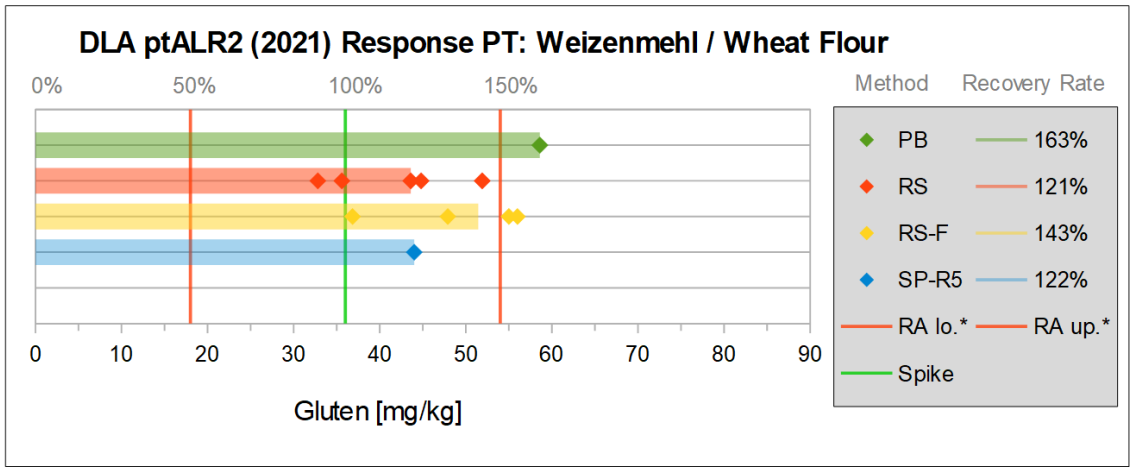


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale gluten content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



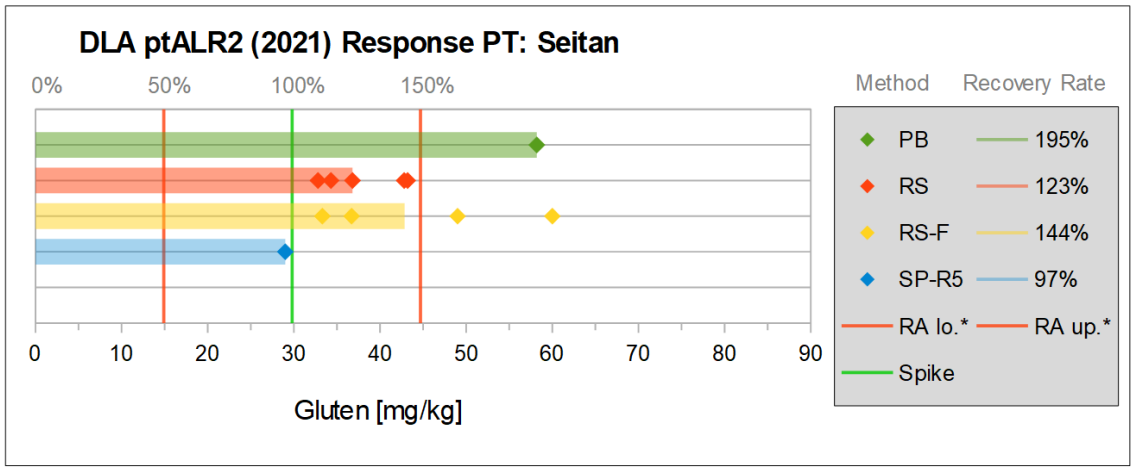


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale gluten content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.2 Participant z-Scores: overview table

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Gluten					PCR Gluten-containing Cereals				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	-1,8	1,3	-2,4	1,8	1,8					
2a	0,12	1,6	-0,94	2,1	2,6					
2b	0,00	-0,2	-2,8	0,89	-0,11					
3										
4	-1,5	1,6	-1,6	1,3	0,47					
5										
6	0,12	1,7	-1,6	2,2	4,1					
7	-1,4	1,4	-1,8	-0,35	0,40					
8	-1,6	0,8	-2,0	0,98	0,94					
9	-1,5	0,2	-2,0	0,09	0,93					
10	-1,8	1,8	-1,4	0,84	0,60					
11	0,15	3,4	-1,3	2,5	3,8					
12	-0,87	2,1	-2,3	-0,04	1,7					

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

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 ELISA-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	
PB	11	05.11.2021	positive	34.2	positive	80.0	positive	20.2	positive	58.6	positive	58.2	negative	<LOQ	2.5	5	30%	Gluten
RS	1	09.11.21	positive	17,8	positive	58,1	positive	12,3	positive	51,9	positive	43,2	negative		5	5		Gluten
RS	3	10.01.2022	positive		positive		positive		positive		positive		negative		0,5			Please select!
RS	7	08.11.21	positive	21,54	positive	58,61	positive	16,91	positive	32,81	positive	32,8	negative	<5	1	5		Gluten
RS	8	25.10.	positive	19,7	positive	52	positive	14,8	positive	44,8	positive	36,8	negative	< 5,00	1	< 5,00		Gluten
RS	10	22.10.21	positive	18,1	positive	63,3	positive	19,2	positive	43,6	positive	34,3	negative		5	5	33,8	Gluten
RS	12		positive	25,8	positive	66,6	positive	13	positive	35,6	positive	42,8	negative	<5				Gluten
RS-F	2a	12.10.	positive	34	positive	61	positive	23	positive	55	positive	49	negative	<5	<3	<5		Gluten
RS-F	4	02.11.21	positive	20,9	positive	61	positive	17,9	positive	47,9	positive	33,3	negative		5	5	50%	Gluten
RS-F	6	16.11.21	positive	34	positive	62	positive	18	positive	56	positive	60	negative		0,5	5	50%	Gluten
RS-F	9	11/26/21	-	20,69	-	45,52	-	15,3	-	36,84	-	36,7	-	< 10	1	10	-	Gluten
SP-R5	2b	12.10.	positive	33	positive	41	positive	9,2	positive	44	positive	29	negative	<3,12	3,12	3,12		Gluten

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Continuation details by participants: ELISA-Methods

Method Abr.	Evalu-ation number	Method	Specificity	Conversion factors for processed products	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	Recalculation from X to Y (factor or %)	e.g. Extraction solution / time / temperature	yes/no	
PB	11	other: prognosis biotech	peroxidase-conjugated antibody against gliadin	-	2.5ml cocktail solution in 50C for 40min, 7.5ml 80%EtOH shaking for 1h, dilution 2.3ml PBS & 200ul extracted sample	no	
RS	1	Ridascreen® Gliadin R7001, R-Biopharm			according to handbook	yes	
RS	3	Ridascreen®, R-Biopharm	Monclonal antibody R5		cocktail solution (50°C 40 min), ethanol 80% (60 min)	yes	
RS	7	Ridascreen® Gliadin R7001, R-Biopharm	Gliadin/Prolamine		asper test-kit instructions	yes	
RS	8	Ridascreen® Gliadin R7001, R-Biopharm			asper test-kit instructions	yes	
RS	10	Ridascreen® Gliadin R7001, R-Biopharm				yes	
RS	12	Ridascreen® Gliadin R7001, R-Biopharm					
RS-F	2a	Ridascreen® FAST Gliadin R7002, R-Biopharm	R5 Mendez, detects Prolamins (Gliadins) from wheat, rye and barley		asper test-kit instructions	yes	
RS-F	4	Ridascreen® Gliadin R7001, R-Biopharm			Extraction with cocktail solution	yes	
RS-F	6	Ridascreen® FAST Gliadin R7002, R-Biopharm	R5 Antibody		asper test-kit instructions, with cocktail solution	yes	
RS-F	9	Ridascreen® FAST Gliadin R7002, R-Biopharm			Sample:0,25g-Extraction: 2,5 ml Extr.Solut.(15 min-60°c)+7,5 ml 80% Ethanol (10 min- 60°C)		
SP-R5	2b	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins	R5 Mendez, detects Prolamins (Gliadins) from wheat, rye and barley		asper test-kit instructions	yes	

5.1.2 PCR-Methods

Method Abr.	Evalu-ation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as peanut
SFA	5		positive		positive		positive		positive		positive		negative		0,4	1		Gluten-containing cereals
SFA-Q	10	06.10.21	positive		positive		positive		positive		positive		negative		0,4			Gluten-containing cereals
div	2	19.10.	positive		positive		positive		positive		positive		negative		5			Wheat-DNA

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Method Abr.	Evalu-ation Number	Method	Specificity	Conversion factors for processed products	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
SFA	5	Sure Food ALLERGEN, R-Biopharm / Congen			CTAB-Extraction+ QIAquick Purification/ RealTime PCR according to Kit instructions CTAB-Extraction+ QIAquick Purification/ RealTime PCR according to Kit instructions	no	
SFA-Q	10	Sure Food Allergen Quant, R-Biopharm / Congen				yes	
div	2	internal Method			/ Realtime PCR / 45 Cycles	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALR2 (2021) Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	71	28,5
2	5,03	81	32,2
3	5,00	93	37,2
4	4,97	80	32,2
5	4,97	92	37,0
6	4,99	79	31,7
7	5,04	88	34,9
8	5,02	69	27,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	81,6	Particles
Standard deviation	8,97	Particles
χ^2 (CHI-Quadrat)	6,90	
Probability	**44**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	32,7	mg/kg
Standard deviation	3,59	mg/kg
rel. Standard deviaton	11,0	%
Horwitz standard deviation	9,5	%
HorRat-value	**1,2**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA ptALR2 (2021) Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	79	31,6
2	5,01	62	24,8
3	4,97	63	25,4
4	5,00	59	23,6
5	4,98	75	30,1
6	5,00	70	28,0
7	5,00	67	26,8
8	5,03	81	32,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,5	Particles
Standard deviation	8,08	Particles
χ^2 (CHI-Quadrat)	6,58	
Probability	**47**	%
Recovery rate	94	%

Normal distribution		
Number of samples	8	
Mean	27,8	mg/kg
Standard deviation	3,23	mg/kg
rel. Standard deviaton	11,6	%
Horwitz standard deviation	9,7	%
HorRat-value	**1,2**	
Recovery rate	94	%

Microtracer Homogeneity Test
DLA ptALR2 (2021) Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	31,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	83	33,3
2	5,05	89	35,2
3	5,02	68	27,1
4	4,97	76	30,6
5	5,04	75	29,8
6	5,00	71	28,4
7	5,05	86	34,1
8	5,01	77	30,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	78,1	Particles
Standard deviation	7,11	Particles
χ^2 (CHI-Quadrat)	4,52	
Probability	**72**	%
Recovery rate	100	%

Normal distribution		
Number of samples	8	
Mean	31,1	mg/kg
Standard deviation	2,83	mg/kg
rel. Standard deviaton	9,10	%
Horwitz standard deviation	9,54	%
HorRat-value	**0,95**	
Recovery rate	100	%

Microtracer Homogeneity Test
DLA ptALR2 (2021) Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	42	16,8
2	5,01	47	18,8
3	5,04	41	16,3
4	4,97	34	13,7
5	5,02	35	13,9
6	5,00	36	14,4
7	5,01	45	18,0
8	5,01	38	15,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	39,7	Particles
Standard deviation	4,72	Particles
χ^2 (CHI-Quadrat)	3,92	
Probability	**79**	%
Recovery rate	76	%

Normal distribution		
Number of samples	8	
Mean	15,9	mg/kg
Standard deviation	1,88	mg/kg
rel. Standard deviaton	11,9	%
Horwitz standard deviation	10,6	%
HorRat-value	**1,1**	
Recovery rate	76	%

Microtracer Homogeneity Test
DLA ptALR2 (2021) Sample 5

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	78	31,0
2	5,03	78	31,0
3	5,05	72	28,5
4	5,05	74	29,3
5	4,97	81	32,6
6	5,01	67	26,7
7	5,03	62	24,7
8	5,01	70	27,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	72,8	Particles
Standard deviation	6,45	Particles
χ^2 (CHI-Quadrat)	4,01	
Probability	**78**	%
Recovery rate	124	%

Normal distribution		
Number of samples	8	
Mean	29,0	mg/kg
Standard deviation	2,57	mg/kg
rel. Standard deviaton	8,87	%
Horwitz standard deviation	9,64	%
HorRat-value	**0,92**	
Recovery rate	124	%

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 ptALR2 - 2021***
PT name	***Response PT Gluten: Processed samples Wheat Flour, Bread, Noodles (Durum Wheat), Bulgur (pre-cooked Wheat) and Seitan (Gluten Protein) in potato powder matrix***
*Sample matrix**	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)*
Number of samples and sample amount	*5 different Samples: 20 g each + 1 "Blank" Sample: 20 g*
Storage	*Samples 1 - 6: room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Gluten / Wheat / Gluten-containing Cereals Samples 1-5: approx. 25 - 150 mg/kg (as total gluten)*
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. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples 1 - 6 and the results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of significant digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest November 26th 2021***
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-Scharf PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Any 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
		SWITZERLAND
		Germany
		USA
		Germany
		Germany
		Germany
		Germany
		Germany
		GREECE
		ITALY
		Germany
		Germany
		ITALY

[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. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. 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
20. 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
21. 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
22. Ministry of Health and Welfare, JSM, Japan 2006
23. 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)
24. 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)

25. 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)
26. 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
27. 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
28. 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
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
32. ASU §64 LFGB L 16.01-9 Untersuchung von Lebensmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016) [Foodstuffs, determination of soya (Glycine max) in cereal flour by real-time PCR]
33. ASU §64 LFGB L 18.00-19 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Sesam (Sesamum indicum) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of sesame (Sesamum indicum) in rice and wheat cookies and sauce powders by PCR]
34. ASU §64 LFGB L 18.00-20 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of almond (Prunus dulcis) in rice and wheat cookies and sauce powders by PCR]
35. ASU §64 LFGB L 18.00-22 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]
36. ASU §64 LFGB L 08.00-66 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Weizen (Triticum L.) und Roggen (Secale cereale) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of wheat (Triticum L.) and rye (Secale cereale) in boiled sausages by real-time PCR]
37. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Informationen 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
38. 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
39. 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]
40. Geisslitz et al. (2019) Comparative Study on Gluten Protein Composition of Ancient (Einkorn, Emmer and Spelt) and Modern Wheat Species (Durum and Common Wheat), Foods 2019 (8): 409 (1-14)
41. Schopf & Scherf (2021) Water Absorption Capacity Determines the Functionality of Vital Gluten Related to Specific Bread, Foods 2021 (10): 228 (1-13)