Evaluation Report
proficiency test

DLA ptALM2 (2021)

ALM-Verification:

Gluten in “gluten-free” Cocoa Biscuit Matrix

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

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

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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 (PT) 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 5-fold of the action level, which is normally based on the threshold value dose (VITAL Concept 3.0) 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 rates for information in the report.
Additionally a quantitative evaluation of the results for the Action Level as well as the Level 5 using z-scores was made for information purposes.

2. Realisation

2.1 Test material

6 PT-samples with the food matrix "gluten-free" cocoa biscuits 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 the sample material is biscuits baked by DLA. The basic composition was identical for all 6 samples (see Table 1).

After crushing and sieving using an impact mill (mesh 1,5 mm) the basic mixture was homogenized and an aliquot was taken from it as blank sample.

To produce the gluten-containing samples, "gluten-free" cocoa biscuits were first baked (150°C, 30 min) with the addition of a wheat flour mixture (further details see below) and then dried (50°C, overnight). Then the gluten-containing cocoa biscuits were crushed and sieved (mesh <1,5 mm) and homogenized.

Afterwards the **spiked sample series** was produced as follows: After crushing and homogenization an aliquot of the gluten containing biscuits was added to the basic mixture. The resulting mixture was homogenized again. Then basic matrix was again added in portions in further steps and homogenized in each case until the total amount was reached.

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 131 % ± 17 % (n=17) in the spiking level sample of the proficiency test DLA ptAL03 (2021) calculated from the ELISA method Ridascreen® Gliadin results.

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „Null“	**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**
Cocoa biscuits, gluten-free (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt	100	99,6	98,0	96,0	90,0	80,0
Cocoa biscuits, spiked (baked 150°C, 30 min) Ingredients: Teff flour (dwarf millet), sugar, margarine (sunflower oil, coconut fat and additives), cocoa powder (4.6%), rice protein, salt, wheat-flour-mixture(25% in dry matter)	–	0,40	2,0	4,0	10,0	20,0
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof Wheat: Wheat flour mixture (21 products from Europe, Asia, USA) – Wheat flour* – *with 10% protein***	–	23,2 2,34	115 11,6	229 23,2	573 57,9	1145 116
– *thereof **Gluten****	–	**2,02**	**9,98**	**20,0**	**49,9**	**99,6**
Extended combined uncertainty (k=2) of Gluten-content (= ± 12 %)		± 0,24	± 1,2	± 2,4	± 6,0	± 12

*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, 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, 18-20].

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 2 to 6 showed a probability of 10%, 84%, 99%, 93% and 25%. 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 1,7, 0,90, 0,66, 0,80 and 1,37 respectively. The values of 1,7 and 1,4 were accepted, because the probabilities of the Poisson distribution were sufficient. The results of the microtracer analysis are given in the documentation.

Additionally the homogeneity of one level was tested by Gluten-ELISA (s. Fig. 1) and with a standard deviation between the samples of <15% is considered to be given for the method used (result: 7,71 mg / kg ± 071, Morinaga ELISA Kit II).

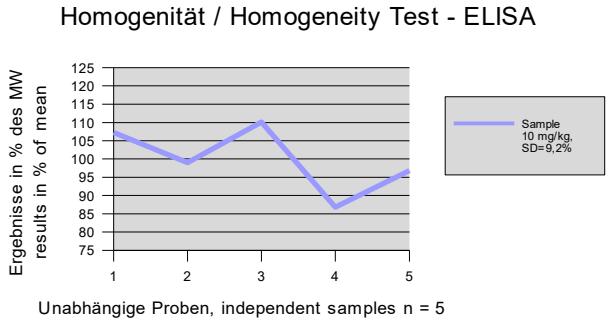


Abb./Fig. 1: Homogeneity test sample 10 mg/kg (level 2)
Results shown as relative percentage of arithmetic mean

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. 0,08 (20,6°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

The portions of test material (sample 1 to 6) were sent to every participating laboratory in the 20th week of 2021. The testing method was optional. The tests should be finished at July 2nd 2021 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 as well as a „blank sample" in the matrix "gluten-free" biscuit with cocoa.

- *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 2.0/3.0), judgement value of the German Commission ALTS/ALS or according to EU Regulation 828/2014).*
- *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 5.2 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.
Queried and documented were the indicated results and details of the test methods like specificity, limit of quantification, 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 at least for one method 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 [32-35]. 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)[21-24, 33]

Allergen	Threshold dose * (Vital Concept 3.0, 2019)		Judgement value ALTS/ALS (2020)		Legislative Maximum value for declaration
	Protein mg/kg	Food mg/kg	Protein mg/kg	Food mg/kg	mg/kg
Egg (as whole egg powder)	2	4,4	> 2	> 4,4	
Milk (as defatted milk powder)	2	5,6	> 2	> 5	
Fish (Finfish, fresh)	13	65	> 13	> 50 (steamed)	
Crustaceans (Shrimps, cooked)	250	1100	> 250	> 2100 (Shrimps, cooked)	
Peanut	2	8	> 2	> 5	
Lupin	26	65	> 26	> 50	
Soy (as Soyflour)	5	13	> 5	> 10	
Cashew / Pistachio	0,5	2,6	> 1	> 5	
Hazelnut and other Tree Nuts (Almond, Brazil Nut, Macadamia)	1	6,4 (4-10)	> 1	> 5	
Walnut / Pecan	0,3		> 1	> 5	
Celery Seed	0,5	-	> 0,5	> 10	
Mustard Seed	0,5	1,9	> 0,5	> 2	
Sesame, unpeeled	1	5,9	> 1	> 5	
Wheat	7	70	> 7	> 100 (>5 Gluten)	**20 (Gluten)****

* calculated by threshold dose considering an intake of 100 g food, protein contents from [22] or nutritional tables Souci/Fachmann/Kraut [22,23, 24]
** Maximum value for declaration as „gluten free" according to EU-VO 828/2014 [21]

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 [30]. 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 experiments

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 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[37-38].

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 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} [39, 40, 41, 43, 47]

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 [29], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [26-28], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [30] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [25].

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 [25-30]	**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 [25]	**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 were calculated according 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.
The **qualitative and quantitative evaluations** were done **separately** for ELISA and PCR methods. 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 harmonizes 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 – 2,0 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]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	Number in RA**		

* RR = Recovery Rate

4.1 Proficiency Test Gluten

4.1.1 Qualitativ: Action Level Matrix-Scores

4.1.1.1 ELISA-Methods

Evaluation number	Level 0 „Null“	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 recorded Level 1 – 5		
6	negative	positive	positive	positive	positive	positive	5 (100%)	AQ-G12	
10	negative	positive	positive	positive	positive	positive	5 (100%)	IL	
1	negative	positive	positive	positive	positive	positive	5 (100%)	RS	Level 2 <5 mg/kg
2a	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
3	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
4	negative	positive	positive	positive	positive	positive	5 (100%)	RS	Level 2 <5 mg/kg
5	negative	negative	positive	positive	positive	positive	4 (80%)	RS	
8	negative	positive	positive	positive	positive	positive	5 (100%)	RS	
2b	negative	negative	positive	positive	positive	positive	4 (80%)	SP-R5	
9	negative	positive	positive	positive	positive	positive	5 (100%)	VT-R5	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	6	10	10	10	10
Number negative	10	4	0	0	0	0
Percent positive	0	60	100	100	100	100
Percent negative	100	40	0	0	0	0
Consensus value	negative	none	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

AQ-G12 = AgraQuant, RomerLabs

IL = Immunolab

RS = Ridascreen®, R-Biopharm

SP-R5 = SensiSpec INgezim Gluten R5, Eurofins

VT-R5 = Veratox, Neogen

Comments:

All participants successfully detected level 2 and thus half of the gluten content of the action level in the processed cocoa biscuits matrix. The lowest level of 2 mg/kg (1/10 of the action level) was detected by 60% of the participants. This value is in the range or below the limits of quantification of the methods.

4.1.1.2 PCR-Methods

Evaluation number	Level 0 „Null“	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 recorded Level 1 – 5		
5	negative	positive	positive	positive	positive	positive	5 (100%)	SFA	
7	negative	positive	positive	positive	positive	positive	5 (100%)	SFA	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comments:

Two participants analyzed gluten or cereals containing gluten using PCR methods. In addition to the action level of 20 mg/kg, all other levels were also successfully detected by both participants.

4.1.2 Quantitative: Recovery Scores and z-Scores

4.1.2.1 ELISA-Results

Evaluation number	Level 1 – 2,0 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]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	Number in RA **		
6	1,40	**69**	-1,2	7,20	**72**	-1,1	15,5	**78**	-0,89	38,4	**77**	-0,92	74,3	**75**	-1,0	5/5 (100%)	AQ-G12	
10	1,20	**59**	-1,6	6,20	**62**	-1,5	18,0	**90**	-0,39	45,0	**90**	-0,39	95,0	**95**	-0,18	5/5 (100%)	IL	
1	<5			10,2	**102**	0,09	24,0	**120**	0,81	66,0	**132**	1,3	123	**124**	0,95	4/4 (100%)	RS	
2a	<5			6,70	**67**	-1,3	15,0	**75**	-0,99	22,0	44	-2,2	82,0	**82**	-0,71	3/4 (75%)	RS	
3	<5			11,5	**115**	0,61	22,6	**113**	0,53	45,7	**92**	-0,34	107	**107**	0,30	4/4 (100%)	RS	
4	<5			10,2	**102**	0,09	20,0	**100**	0,01	50,7	**102**	0,06	103	**103**	0,14	4/4 (100%)	RS	
5				8,30	**83**	-0,67	16,6	**83**	-0,67	42,7	**86**	-0,58	90,4	**91**	-0,37	4/4 (100%)	RS	
8	2,25	**112**	0,46	11,1	**111**	0,45	24,1	**121**	0,83	38,3	**77**	-0,93	114	**114**	0,58	4/4 (100%)	RS	
2b	<3,12			6,90	**69**	-1,2	13,0	**65**	-1,4	31,0	**62**	-1,5	66,0	**66**	-1,3	4/4 (100%)	SP-R5	
9	1,90	**94**	-0,23	7,97	**80**	-0,80	15,8	**79**	-0,84	34,5	**69**	-1,2	84,7	**85**	-0,60	5/5 (100%)	VT-R5	

RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %	
Number in RA	**4**	**0**	Number in RA	**10**		Number in RA	**10**		Number in RA	**9**		Number in RA	**10**	
Percent in RA	**100**	**0**	Percent in RA	**100**		Percent in RA	**100**		Percent in RA	**90**		Percent in RA	**100**	

* Recovery rate 100% Reference value: Gluten, s. Page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:

AQ-G12 = AgraQuant, RomerLabs

IL = Immunolab

RS = Ridascreen®, R-Biopharm

SP-R5 = SensiSpec INgezim Gluten R5, Eurofins

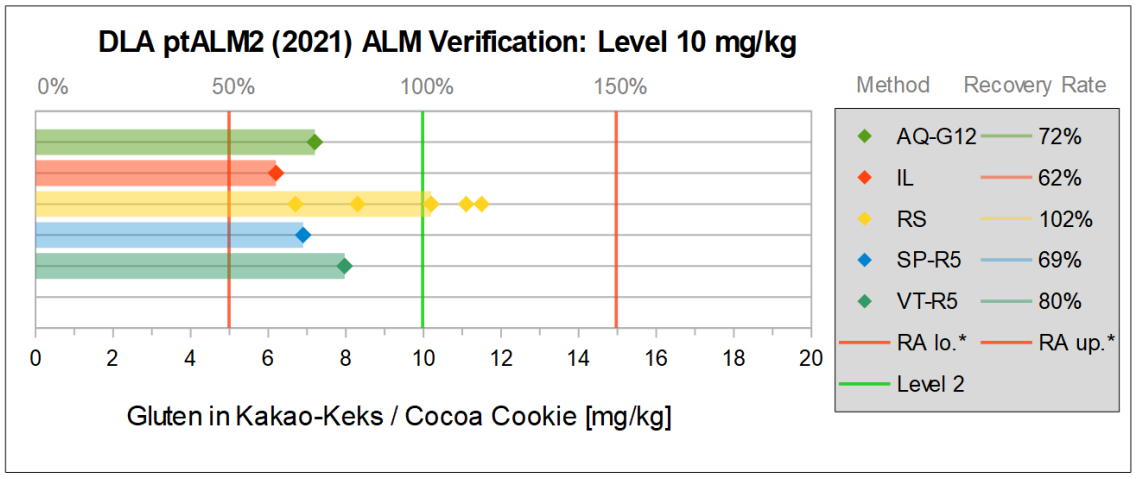
VT-R5 = Veratox, Neogen

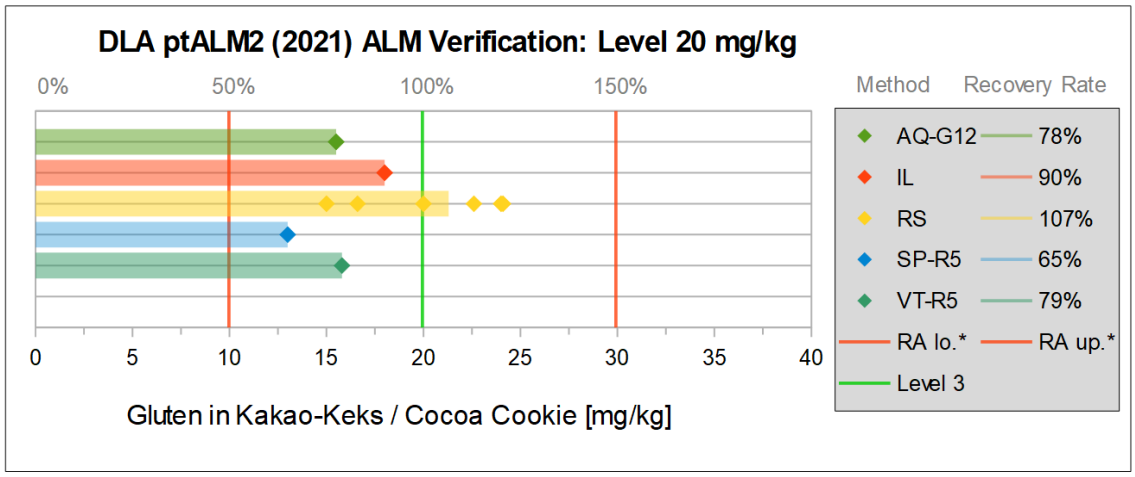
Comments:

With one exception, all recovery rates of the participant results for the levels 1 to 5 were in the range of the AOAC recommendations of 50-150%.

4.1.2.2 PCR-Results

No quantitative results were available for the PCR methods.





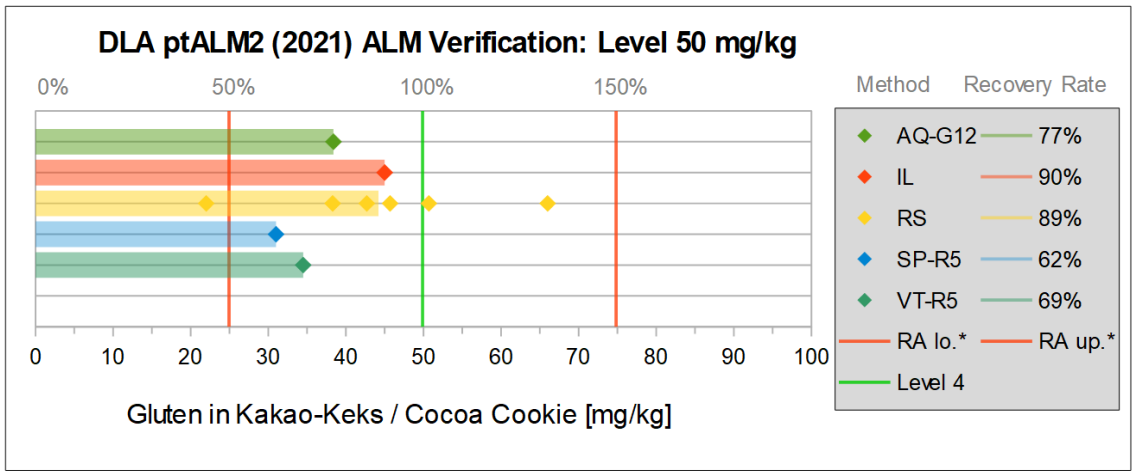


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale gluten content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.1.3 Informative Data: Statistical characteristics gluten

4.1.3.1 ELISA-Methods

Sample: Level 10,0 mg/kg

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{METHOD\ RS}}}$
Number of results	10	6
Number of outliers	0	0
Mean	8,63	9,67
Median	8,14	10,2
Robust Mean (X_{pt})	**8,63**	**9,67**
Robust standard deviation (S*)	**2,22**	**2,07**
Target range:		
Target standard deviation σ_{pt}	**2,16**	**2,42**
lower limit of target range	**4,31**	**4,83**
upper limit of target range	**12,9**	**14,5**
Quotient S/σ_{pt}*	*1,0*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*0,877*	*1,06*
Results in the target range	*10*	*6*
Percent in the target range	*100*	*100*

Methods:
RS = R-Biopharm, Ridascreen®

Comments on the statistic data:

Assigned values were the robust means of the results of all methods or method RS.

The calculation of the z-scores was based on a target standard deviation of 25% (see Fig. 3, p. 23).

All data are for information only.

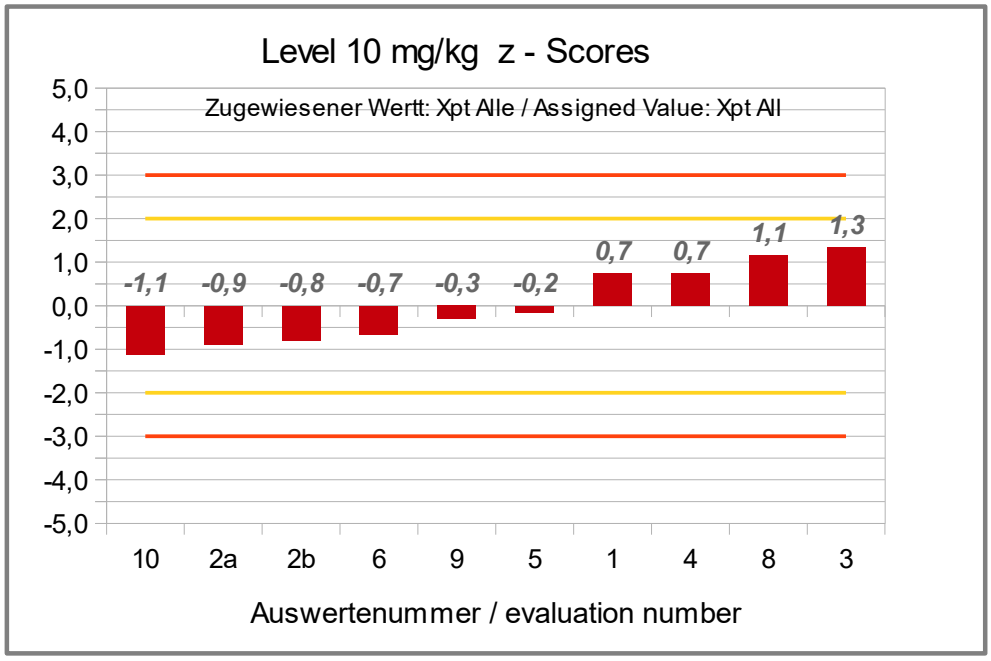


Abb./Fig. 3:
z-Scores level 10,0 mg/kg (ELISA-results as gluten)
Assigned value: robust mean (alg. A) of all results

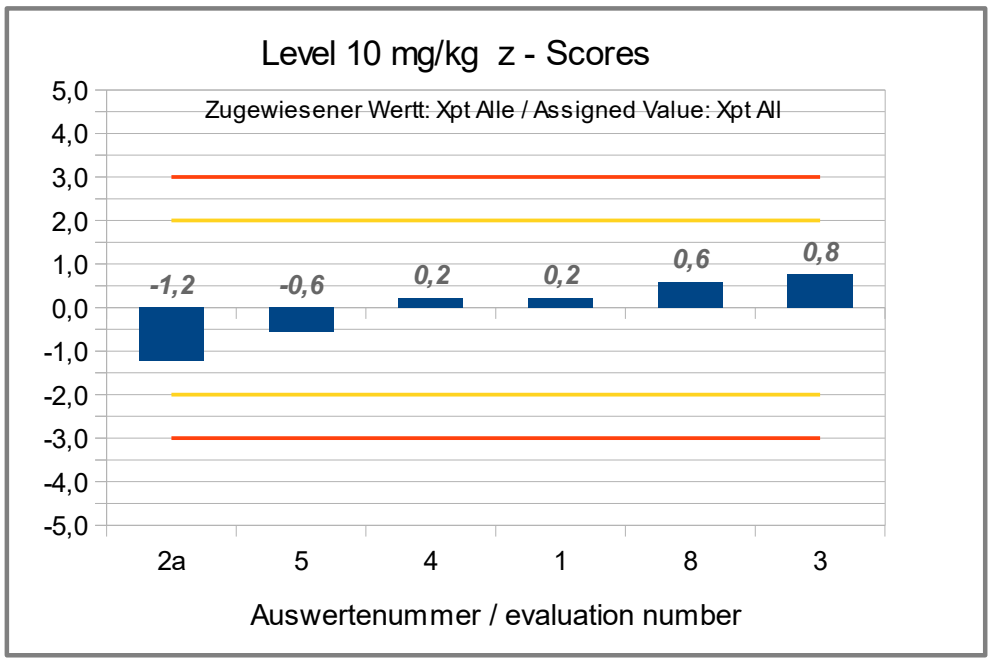


Abb./Fig. 4:
z-Scores level 10,0 mg/kg (ELISA-results as gluten)
Assigned value: robust mean (alg. A) of results of method RS (R-Biopharm, Ridascreen)

Sample: Action Level 20,0 mg/kg

Statistic Data	**All Results** *[mg/kg]*	**Method RS** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS}}$
Number of results	10	6
Number of outliers	0	0
Mean	18,5	20,4
Median	17,3	21,3
Robust Mean (X_{pt})	**18,5**	**20,4**
Robust standard deviation (S*)	**4,53**	**4,40**
Target range:		
Target standard deviation σ_{pt}	**4,61**	**5,10**
lower limit of target range	**9,23**	**10,2**
upper limit of target range	**27,7**	**30,6**
Quotient S/σ_{pt}*	*0,98*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*1,79*	*2,24*
Results in the target range	*10*	*6*
Percent in the target range	*100*	*100*

Methods:
RS = R-Biopharm, Ridascreen®

Comments on the statistic data:

Assigned values were the robust means of the results of all methods or method RS.

The calculation of the z-scores was based on a target standard deviation of 25% (see Fig. 3, p. 23).

All data are for information only.

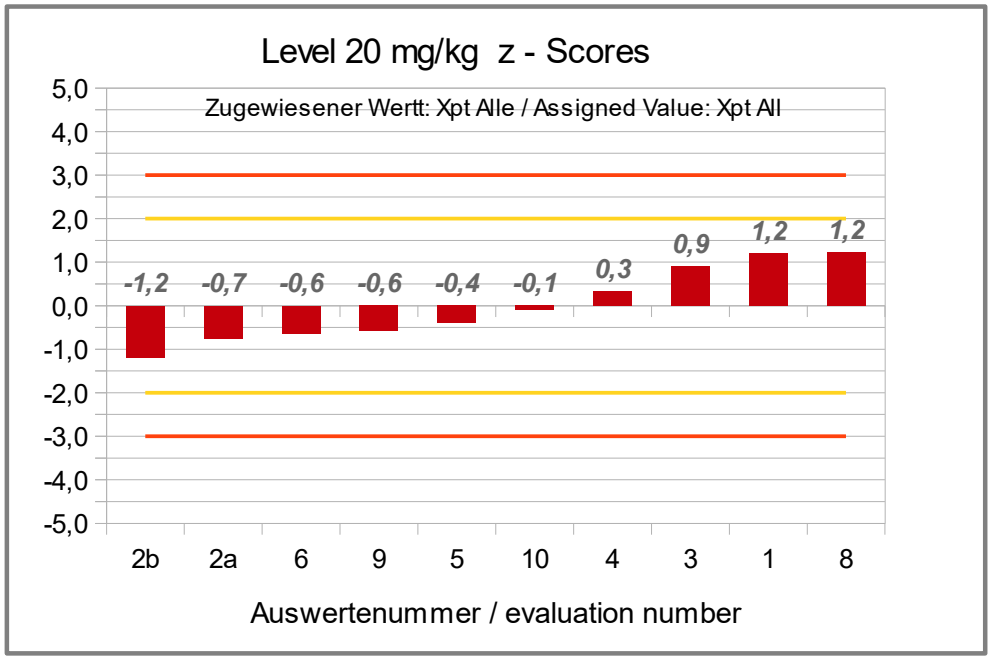


Abb./Fig. 5:
z-Scores action level 20,0 mg/kg (ELISA-results as gluten)
Assigned value: robust mean (alg. A) of all results

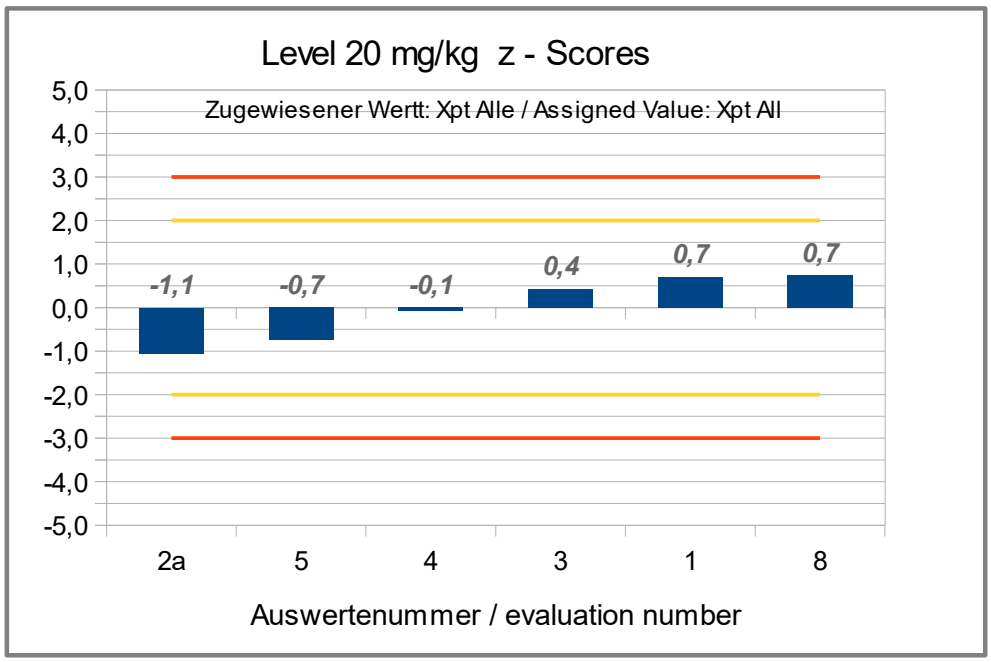


Abb./Fig. 6:
z-Scores action level 20,0 mg/kg (ELISA-results as gluten)
Assigned value: robust mean (alg. A) of results of method RS (R-Biopharm, Ridascreen)

4.1.3.2 PCR-Methods

There were no quantitative results by PCR methods submitted, thus no quantitativ evaluation was done.

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				
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 1	Level 2	Level 3	Level 4	Level 5
1		0,09	0,81	1,3	0,95					
2a		-1,3	-1,0	-2,2	-0,71					
2b		-1,2	-1,4	-1,5	-1,3					
3		0,61	0,53	-0,34	0,30					
4		0,09	0,01	0,06	0,14					
5		-0,67	-0,67	-0,58	-0,37					
6	-1,2	-1,1	-0,89	-0,92	-1,0					
7										
8	0,46		0,83	-0,93	0,58					
9	-0,23	0,45	-0,84	-1,2	-0,60					
10	-1,6	-0,80	-0,39	-0,39	-0,18					

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 and Lateral Flow Methods

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 Level 10 mg/kg		Result Sample 2 Level 100 mg/kg		Result Sample 3 Level 20 mg/kg		Result Sample 4 Level 2 mg/kg		Result Sample 5 Level 50 mg/kg		Result Sample 6 Blank		NWG / LOD *	BG / LOQ *	MU*	Quantitativee Result given as	Method
		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	e.g. Food / Protein	Test-Kit + Provider
AQ-G12	6	06.08.21	-	7,2	-	74,3	-	15,5	-	1,4	-	38,4	-	0	2	4		Gluten	AgraQuant ELISA Gluten G12 COKAL0200, RomerLabs
IL	10	07.07.22	positive	6,2	positive	95	positive	18	positive	1,2	positive	45	negative	0	0,6	4		Gluten	Immunolab Gliadin/Gluten ELISA
RS	1	20.05.	positive	10,2	positive	123,2	positive	24	positive	<5	positive	66	negative	<1	1	5	20	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	2a	19.05.21	positive	6,7	positive	82	positive	15	negative	<5	positive	22	negative	<5	3	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	3	07/06-18/06	positive	11,5	positive	107	positive	22,6	negative	< 5	positive	45,7	negative	< 5	5	5	0,3	Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	4	18.06.21	positive	10,2	positive	103	positive	20	positive	< 5	positive	50,7	negative		1	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	5	07.06.21	positive	8,3	positive	90,4	positive	16,6	negative		positive	42,7	negative		5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
RS	8	29.06.	positive	11,1	positive	114	positive	24,1	positive	2,25	positive	38,3	negative	< 0,625	0,5	5		Gluten	Ridascreen® Gliadin R7001, R-Biopharm
SP-R5	2b	20.05.21	positive	6,9	positive	66	positive	13	negative	<3,12	positive	31	negative	<3,12	3,12	3,12		Gluten	SENSISpec Ingezim Gluten R5 30.GLU.K2, Eurofins
VT-R5	9	10.06.21	-	7,97	-	84,66	-	15,78	-	1,9	-	34,46	-	0				Gluten	Veratox Gliadin R5, Neogen

* 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 Abbr.	Evaluation number	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Antibody	e.g. Extraction solution / Time / Temperature	yes/no	
AQ-G12	6			yes	
IL	10	Gliadin	using ILE-EXSCH2 extraction additive		
RS	1	Gliadin from wheat (and prolamins from rye, barley, spelt)	Coctail solution 40 min at 50°C, then EtOH (60%) 1hr at RT. 80 µl clear extract w ith dilution buffer ad 1000 µl, 100 µl in test in duplicate	yes	
RS	2a	R5 antibody from Mendez detects prolamins (Gliadins) from wheat, rye and barley	as per kit instructions	yes	
RS	3		Cocktail-solution	yes	
RS	4	Gliadin	in ELISA R5-antibodies used		Sample 4: Extinctions clearly higher than negative controls, but < LOQ
RS	5			yes	Sample 4: higher OD, but < 5 mg/kg
RS	8		as per kit instructions	yes	Standard 2 (5mg/kg) w as diluted to 0,625mg/kg, thus LOQ w as decreased to < 0,625mg Gluten/kg. A clear extinction difference betw een Std. 1 (0mg/kg) and Std. 2 (0,625mg/kg) w as observed.
SP-R5	2b	R5 antibody from Mendez detects prolamins (Gliadins) from wheat, rye and barley	as per kit instructions	yes	
VT-R5	9				

5.1.2 PCR-Methods

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 Level 10 mg/kg		Result Sample 2 Level 100 mg/kg		Result Sample 3 Level 20 mg/kg		Result Sample 4 Level 2 mg/kg		Result Sample 5 Level 50 mg/kg		Result Sample 6 Blank		NWG / LOD *	BG / LOQ *	MU*	Quantitativee Result given as	Method
		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	e.g. Food / Protein	Test-Kit + Provider
SFA	5	14.05.21	positive		positive		positive		positive		positive		negative					Gluten	Sure Food Allergen Quant, R-Biopharm / Congen
SFA	7		positive	10	positive	100	positive	20	positive	2	positive	50	negative	0	0,4	1		Gluten-containing cereals	Sure Food ALLERGEN, R-Biopharm / Congen

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

Continuation details by participants: PCR-Methods

Method Abk.	Evaluation-number	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
SFA	5			yes	
SFA	7	DNA gluten-containing cereals	CTAB/ QIAquick Purification/ RealTime PCR	no	Quantitative results not determined. Merely assigned to specified values according to the determined Ct values.

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA-ptALM2 (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	52,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,55	70	54,9
2	2,52	93	73,8
3	2,50	96	76,8
4	2,54	70	55,1
5	2,51	69	55,0
6	2,47	85	68,8
7	2,55	81	63,5
8	2,45	91	74,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	82,0	Particles
Standard deviation	11,81	Particles
χ^2 (CHI-Quadrat)	11,91	
Probability	**10**	%
Recovery rate	125	%

Normal distribution		
Number of samples	8	
Mean	65,3	mg/kg
Standard deviation	9,41	mg/kg
rel. Standard deviaton	14,4	%
Horwitz standard deviation	8,53	%
HorRat-value	**1,7**	
Recovery rate	125	%

Microtracer Homogeneity Test
DLA-ptALM2 (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	34,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,95	62	25,1
2	5,00	70	28,0
3	5,01	67	26,7
4	5,00	63	25,2
5	4,96	53	21,4
6	5,01	68	27,1
7	5,00	57	22,8
8	5,00	64	25,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	63,0	Particles
Standard deviation	5,55	Particles
χ^2 (CHI-Quadrat)	3,42	
Probability	**84**	%
Recovery rate	74	%

Normal distribution		
Number of samples	8	
Mean	25,2	mg/kg
Standard deviation	2,22	mg/kg
rel. Standard deviaton	8,81	%
Horwitz standard deviation	9,84	%
HorRat-value	**0,90**	
Recovery rate	74	%

Microtracer Homogeneity Test
DLA-ptALM2 (2021) Sample 3

Weight whole sample	1,20	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	40,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,50	56	44,8
2	2,53	60	47,4
3	2,49	53	42,6
4	2,52	55	43,7
5	2,51	56	44,6
6	2,51	51	40,6
7	2,51	61	48,6
8	2,49	53	42,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	55,6	Particles
Standard deviation	3,30	Particles
χ^2 (CHI-Quadrat)	1,37	
Probability	**99**	%
Recovery rate	109	%

Normal distribution		
Number of samples	8	
Mean	44,4	mg/kg
Standard deviation	2,63	mg/kg
rel. Standard deviaton	5,93	%
Horwitz standard deviation	9,04	%
HorRat-value	**0,66**	
Recovery rate	109	%

Microtracer Homogeneity Test
DLA-ptALM2 (2021) Sample 4

Weight whole sample	0,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	68,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,46	74	60,2
2	2,52	80	63,5
3	2,51	68	54,2
4	2,50	70	56,0
5	2,51	67	53,4
6	2,51	65	51,8
7	2,51	74	59,0
8	2,50	74	59,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	71,5	Particles
Standard deviation	4,96	Particles
χ^2 (CHI-Quadrat)	2,40	
Probability	**93**	%
Recovery rate	84	%

Normal distribution		
Number of samples	8	
Mean	57,1	mg/kg
Standard deviation	3,96	mg/kg
rel. Standard deviaton	6,93	%
Horwitz standard deviation	8,70	%
HorRat-value	**0,80**	
Recovery rate	84	%

Microtracer Homogeneity Test
DLA-ptALM2 (2021) Sample 5

Weight whole sample	0,50	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	87,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,47	83	67,2
2	2,49	112	90,0
3	2,51	99	78,9
4	2,49	83	66,7
5	2,51	107	85,3
6	2,51	100	79,7
7	2,51	111	88,4
8	2,51	108	86,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	100,3	Particles
Standard deviation	11,35	Particles
χ^2 (CHI-Quadrat)	8,99	
Probability	**25**	%
Recovery rate	92	%

Normal distribution		
Number of samples	8	
Mean	80,3	mg/kg
Standard deviation	9,08	mg/kg
rel. Standard deviaton	11,3	%
Horwitz standard deviation	8,27	%
HorRat-value	**1,37**	
Recovery rate	92	%

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 ptALM2 - 2021***
PT name	***ALM-Verification Gluten 5 Samples containing Wheat Flour in "gluten-free" Cocoa Biscuits*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Cocoa biscuits (baked: 150 ° C, 30 min) / Ingredients: teff flour (dwarf millet), sugar, margarine, rice protein, cocoa powder, salt and allergenic food wheat flour (except "blank sample")*
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), 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 July 02nd 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
		Germany
		Germany
		Germany
		ITALY
		Germany
		Germany
		Germany
		Germany
		USA
		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

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