Evaluation Report
proficiency test

DLA ptALM1 (2020)

ALM-Verification:

Mustard in Spice-Mixture

5 Samples with Mustard *(Sinapis alba)* (levels: 0,5 / 2,5 / 5,0 / 12,5 / 25 mg/kg)

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General Information on the proficiency test (PT)

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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), 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 2.0) or the assessment values of the ALTS/ALS (German Food Expert Committee) (see Table 3). The evaluation of PT-results was performed qualitative in scores from 1-5 (Score 3 = Action Level successfully detected). Quantitative results were given including the recovery 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 spice mixture were provided for qualitative detection and optional quantitative determination of mustard *(Sinapis alba)*. The mustard levels of the PT-sample series were in the range from 0,5 mg/kg to 25 mg/kg, whereas the medial level represents the "Action Level" (see Table 1).

The food matrix of sample material was a mixture of ground spices (powder). The basic composition was identical for all 6 samples (see Table 1).
After homogenization of the basic mixture an aliquot was taken from it as blank sample.

For preparation of the mustard containing allergen-premixes the raw materials were crushed and sieved by a centrifugal mill (mesh 500 µm) and homogenized.
Afterwards the **spiked sample series** was produced as follows: After crushing and homogenization an aliquot of the mustard premix was added to the basic mixture. The resulting mixture was homogenized again. Afterwards basic mixture was added stepwise (2-3 steps) including homogenization after each step until the total amount of sample material was reached.

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

For the spiking a mixture of yellow mustard seeds *(Sinapis alba)* from a total of 9 products (from Germany, East Europe and India) was used.
For the samples (matrix: potato powder / maltodextrin) of proficiency test DLA ptALR1 (2020) this mixture gave a mean recovery rate for mustard about 155 % ± 39 % (n=10) calculated from different ELISA methods* and of 134 % ± 21 % (n=6) calculated from the ELISA method RS-F**.

* div. ELISA methods = AgraQuant (Romer Labs), Ridascreen® Fast (R-Biopharm), SensiSpec (Eurofins), Veratox (Neogen)
** ELISA method RS-F = Ridascreen® Fast (R-Biopharm)

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „blank“	**Level 1** 0,5 mg/kg	**Level 2** 2,5 mg/kg	**Level 3** 5,0 mg/kg	**Level 4** 12,5 mg/kg	**Level 5** 25 mg/kg
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Spice Mixture Ingredients: Paprika, turmeric, pepper, onions, marjoram, caraway seeds, chillies, garlic	100	>99,9	>99,9	>99,9	>99,9	>99,9
Mustard seed *(Sinapis alba)* Mixture of 9 products (Europe, Asia) and other ingredients (maltodextrin, silicon dioxide)	–	0,001	0,005	0,013	0,025	0,050
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof Mustard: – as Mustard* – *with 26,1% protein***	–	**0,505** 0,132	**2,53** 0,659	**5,05** 1,32	**12,6** 3,28	**25,2** 6,58
Extended combined uncertainty (k=2) of mustard content (= ± 11,3 %)		± 0,057	± 0,29	± 0,57	± 1,4	± 2,8

*Allergen contents as „total food“ as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,30 for mustard protein)

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 mustard.
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 Characterization of the PT-Sample series

The PT-sample series was characterized by ELISA (Immunolab Mustard ELISA, with extraction additive). The spiking levels correlated with the ascending values of measured results (see Fig. 1). The recovery rates ranged from 57% to 88% for levels 3-5. Levels 0-2 were estimated below the limit of quantification for information. The relative standard deviation (RSD) of the action level (level 3) was approx. 12,4%.

Table 2: Characterization of the PT-sample series mustard in spice mixture by ELISA determination (Immunolab ELISA).

PT-Sample	Level 0*	Level 1*	Level 2*	Level 3	Level 4	Level 5
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
Spiking	0,0	0,51	2,53	5,05	12,6	25,2
Result 1	0,08	0,39	1,11	3,71	5,77	18,8
Result 2	0,11	0,45	1,07	2,91	6,64	32,5
Result 3	0,46°	0,44	1,27	3,18	8,53	16,6
Result 4	0,17	0,39	1,60	11,3°	7,89	20,6
Mean		0,42	1,26	3,27	7,21	22,1
SD	-	0,03	0,24	0,41	1,24	7,12
RSD [%]	-	8,1	19,4	12,4	17,2	32,2
Recovery [%]	-	83	50	65	57	88

* Level 0-2: values below LOQ °Outliers excluded

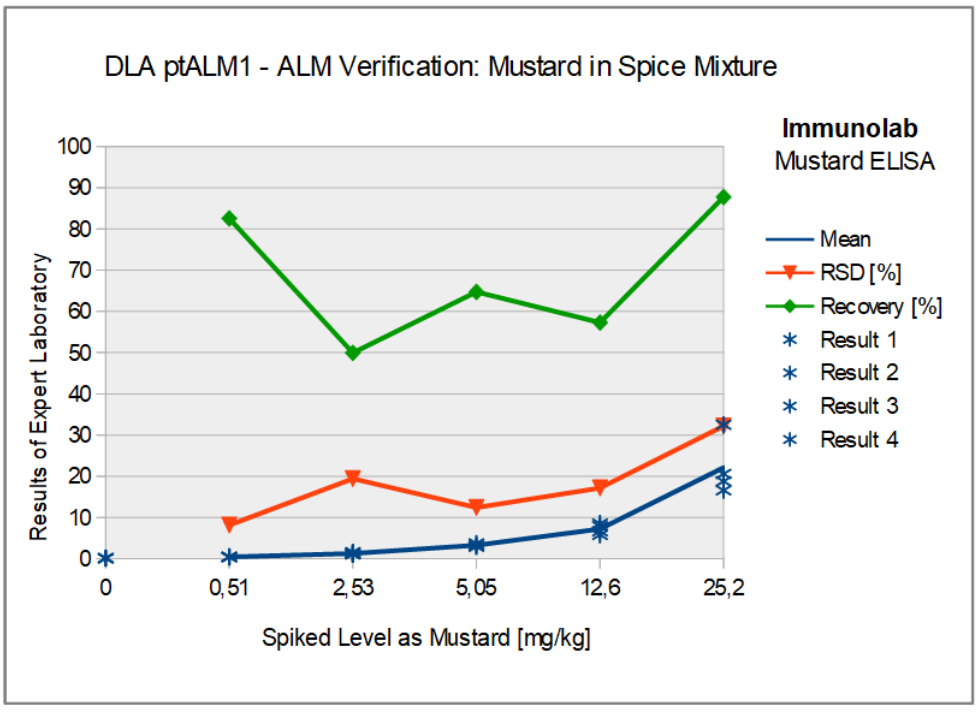


Abb./Fig. 1: ELISA results of PT-sample series mustard in spice mixture (Immunolab ELISA Kit), *Note: the x-scale is not shown linear to obtain a better recognizability of low values.*

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1, 2, 3, 5 and 6 showed a probability of 65%, 15%, 97%, 99% and 98%. 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 0,95, 1,4, 0,70, 0,52 and 0,56 respectively. The value of 1,4 was accepted, because the probability of the Poisson distribution was sufficient. 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. 0,45 (18,7°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 47th week of 2020. The testing method was optional. The tests should be finished at January 29th 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 Mustard as well as a „blank sample" in the matrix Spice Mixture.

- *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 5 mg/kg mustard can be detected in the processed matrix (= Action Level 1 (VITAL concept 2.0) and judgement value of the German Commission ALTS/ALS).*
- *If possible, the indication of quantitative results is desirable in order to compare them with the levels of addition.*

Please note the attached information on the proficiency test.
(see documentation, section 5.2 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.

7 participants submitted results. 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 [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 2.0, 2014)		Threshold dose * (Vital Concept 3.0, 2019)		Judgement value ALTS/ALS	Legislative Maximum value for declaration
	Protein mg/kg	Food mg/kg	Protein mg/kg	Food mg/kg	Food mg/kg	mg/kg
Egg (as whole egg powder)	0,3	0,66	2	4,4	> 1	
Milk (as defatted milk powder)	1	2,8	2	5,6	> 2,5	
Fish (Finfish, fresh)	-	-	13	65	-	
Crustaceans (Shrimps, cooked)	100	440	250	1100	-	
Peanut	2	8	2	8	> 5	
Lupin	40	100	26	65	> 50	
Soy (as Soyflour)	10	25	5	13	> 20	
Cashew / Pistachio	-	-	0,5	2,6	> 50	
Hazelnut and other Tree Nuts (Almond, Brazil Nut, Macadamia)	1	6,4 (4-10)	1	6,4 (4-10)	> 20	
Walnut / Pecan	-	-	0,3		-	
Celery Seed	-	-	0,5	-	> 20	
Mustard Seed	0,5	1,9	0,5	1,9	**> 5**	
Sesame, unpeeled	2	11,8	1	5,9	> 10	
Wheat	10	100	7	70	> 80	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 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 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 12 – 176% for PCR methods (mustard), 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} [40, 41, 43-46]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
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 multiplex 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 multiplex ASU 18.00-22
Mustard, brown / black	Sausage, autoclaved	146,7 50,0 15,8	147 % 125 % 158 %	-	12,3% 17,2% 15,4%	22,0% 31,6% 27,1%	20,2% 29,2% 24,8%	rt-PCR ASU 08.00-64
Mustard, brown / black	Sausage, autoclaved	168,3 52,9 17,6	168 % 132 % 176 %	-	11,4% 10,0% 23,1%	31,6% 23,1% 46,3%	29,5% 21,9% 43,3%	rt-PCR ASU 08.00-65
Mustard, white	Boiled Sausage (100°C, 60min)	79,9 37,0 18,0 8,0	80 % 93 % 90 % 80 %	-	13,6% 15,7% 14,4% 15,4%	23,6% 29,2% 30,6% 26,1%	21,6% 27,0% 28,9% 23,7%	rt-PCR ASU 08.00-59
Mustard, weiß	Boiled Sausage (100°C, 60 min)	103,3 45,9	103 % 115 %	- -	11,8% 14,7%	17,1% 21,8%	14,9% 19,2%	rt-PCR ASU 08.00-65
Mustard, weiß	Sausage, autoclaved	11,7	11,7 %	-	24,1%	34,3%	29,8%	rt-PCR ASU 08.00-65

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 ($U_{(Xpt)}$) [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 proficiency test, all ELISA results were given as total food item (mustard seed), so that no conversions were necessary.

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

Participant	Level 0 „blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 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 – 0,5 mg/kg			Level 2 – 2,5 mg/kg			Level 3 – 5,0 mg/kg (Action Level)			Level 4 – 12,5 mg/kg			Level 5 – 25 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 Mustard

4.1.1 Qualitativ: Action Level Matrix-Scores

4.1.1.1 ELISA-Methods

Evaluation number	Level 0 „Blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 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		
2	negative	negative	negative	negative	positive	positive	2 (40%)	AQ	
1a	negative	negative	positive	positive	positive	positive	4 (80%)	NL	
7	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	
1b	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
6a	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
6b	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
4	negative	positive	positive	positive	positive	positive	5 (100%)	SP	
5	negative	negative	negative	positive	positive	positive	3 (60%)	SP	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	2	6	7	8	8
Number negative	8	6	2	1	0	0
Percent positive	0	25	75	88	100	100
Percent negative	100	75	25	13	0	0
Consensus value	negative	negative	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

AQ = AgraQuant, RomerLabs

NL = nutriLinia® Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

With one exception, all participants successfully detected the action level of 5,0 mg/kg and the higher levels 4 and 5. Level 2 was detected as positive by 75% (6) of the participants and level 1 by only 25% (2) of the participants. According to the test kit manuals, the limits of quantification of the ELISA methods used were 0,5 mg/kg (RS-F) and 2 mg/kg (AQ, NL, SP), respectively.

4.1.1.2 PCR-Methods

Evaluation number	Level 0 „Blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 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	negative	positive	positive	positive	positive	4 (80%)	ASU	
7	negative	positive	positive	positive	positive	positive	5 (100%)	SFA	„blank“ negative (<1 mg/kg)
3a	negative	positive	positive	positive	positive	positive	5 (100%)	SFA	„blank“ negative (1 mg/kg)
3b	negative	positive	positive	positive	positive	positive	5 (100%)	SFA-4p	„blank“ negative (1 mg/kg)
2	positive	positive	positive	positive	positive	positive	5 (100%)	SFA-Q	„blank“ positive (0,1 mg/kg)

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	1	4	5	5	5	5
Number negative	4	1	0	0	0	0
Percent positive	20	80	100	100	100	100
Percent negative	80	20	0	0	0	0
Consensus value	negative	negative	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

Comments:

Four participants detected the action level of 5,0 mg/kg as well as the lower level 2 and the higher levels 4 and 5. Level 1 was detected by three of the participants. One participant applied two different methods.
For the "blank sample" a positive result with a content of 0,1 mg/kg was given, while participant 3 classified the "blank sample" as negative with a content of 1 mg/kg.

4.1.2 Quantitative: Recovery Scores and z-Scores

4.1.2.1 ELISA-Results

	Level 1 – 0,5 mg/kg			Level 2 – 2,5 mg/kg			Level 3 – 5,0 mg/kg (Action Level)			Level 4 – 12,5 mg/kg			Level 5 – 25 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 **		
2	< 2			< 2			< 2			6,80	**54**	-1,8	11,6	46	-2,2	1/5 (20%)	AQ	
1a	0			1,60	**63**	-1,5	1,75	35	-2,6	2,90	23	-3,1	3,64	14	-3,4	1/5 (20%)	NL	
7	0,33	**65**	-1,4	0,76	30	-2,8	1,85	37	-2,5	3,93	31	-2,8	7,31	29	-2,8	1/5 (20%)	RS-F	
1b	0			1,60	**63**	-1,5	1,30	26	-3,0	2,90	23	-3,1	5,40	21	-3,1	1/5 (20%)	RS-F	
6a	<0,50																RS-F	without extraction additive
6b				4,08	162	2,5	8,99	178	3,1	>13,5			6,50	26	-3,0	0	RS-F	with extraction additive
4	0,42	**83**	-0,67	1,26	**50**	-2,0	3,27	**65**	-1,4	7,21	**57**	-1,7	21,98	**87**	-0,51	5/5 (100%)	SP	with extraction additive
5	<2			<2			<2			3,50	28	-2,9	5,60	22	-3,1	0	SP	

RR**	50-150 %	RR**	50-150 %	RR**	50-150 %	RR**	50-150 %	RR**	50-150 %
Number in RA	**2**	Number in RA	**3**	Number in RA	**1**	Number in RA	**2**	Number in RA	**1**
Percent in RA	**100**	Percent in RA	**60**	Percent in RA	**20**	Percent in RA	**33**	Percent in RA	**14**

Methods:
AQ = AgraQuant, RomerLabs
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

** Acceptance range of AOAC for allergen ELISAs

Comments:
With one exception (results no. 4), only very few recovery rates of the participant results for levels 1 to 5 were in the range of the AOAC requirements of 50-150%.

4.1.2.2 PCR-Results

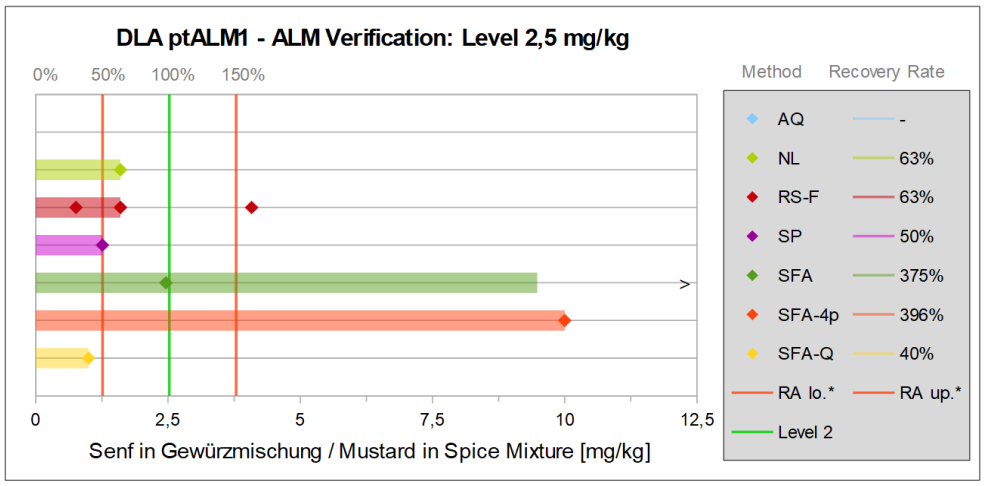
Evaluation number	Level 1 – 0,5 mg/kg			Level 2 – 2,5 mg/kg			Level 3 – 5,0 mg/kg (Action Level)			Level 4 – 12,5 mg/kg			Level 5 – 25 mg/kg			RR-Score	Method	Remarks
	Result	RR *		Result	RR *		Result	RR *		Result	RR *		Result	RR *		RR *		
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	Number in RA **		
5																	ASU	
7	0,56	**111**	0,44	2,46	**97**	-0,10	5,04	**100**	0,00	12,1	**96**	-0,15	23	**92**	-0,32	5/5 (100%)	SFA	
3a	11,0	2178	83	16,5	653	22	37,5	743	26	76,0	604	20	133	528	17	0	SFA	
3b	7,00	1386	51	10,0	396	12	17,5	347	9,9	31,0	246	5,9	76	302	8,1	0	SFA-4p	
2	0,20	40	-2,4	1,00	40	-2,4	3,00	**59**	-1,6	13,6	**108**	0,32	7,3	29	-2,8	2/5 (40%)	SFA-Q	
	RR**	50-150 %		RR**	50-150 %		RR**	50-150 %		RR**	50-150 %		RR**	50-150 %				
	Number in RA	1	2	Number in RA	1		Number in RA	2		Number in RA	2		Number in RA	1				
	Percent in RA	25	50	Percent in RA	25		Percent in RA	50		Percent in RA	50		Percent in RA	25				

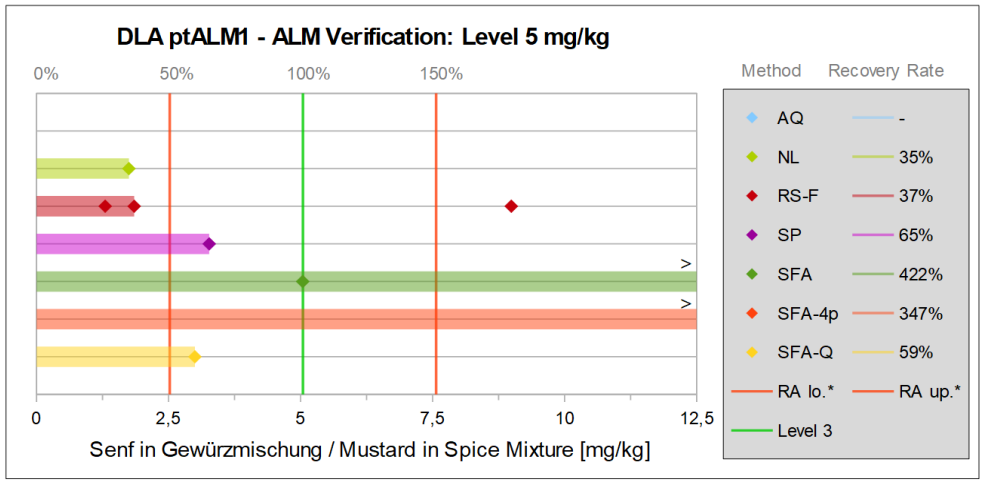
Methods:
ASU = ASU §64 Methode/method
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Cong
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Cong

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

** Acceptance range of AOAC for allergen ELISAs

Comments:
With one exception (results no. 7), only very few recovery rates of the participant results for levels 1 to 5 were in the range of the AOAC requirements of 50-150%.





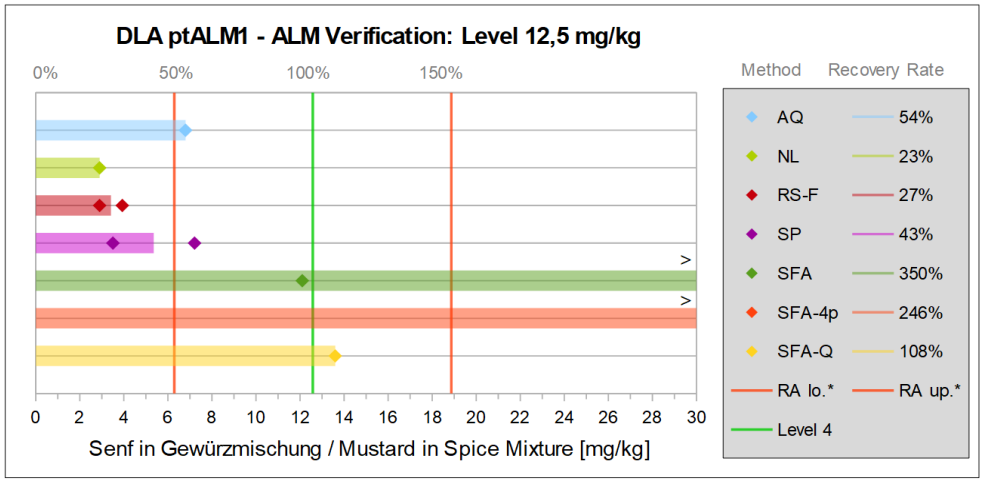


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale mustard content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.1.3 Informative Data: Statistical characteristics mustard

4.1.3.1 ELISA-Methods

Sample: Level 12,5 mg/kg

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	4,54
Median	3,72
Robust Mean (X_{pt})	**4,54**
Robust standard deviation (S*)	**2,21**
Target range:	
Target standard deviation $\sigma_{pt'}$	**1,60**
lower limit of target range	**1,34**
upper limit of target range	**7,74**
Quotient S/$\sigma_{pt'}$*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*1,13*
Results in the target range	*6*
Percent in the target range	*100*

Comments on the statistic data:

Assigned value was the robust mean of the results of all methods.

The calculation of the z-scores was based on a target standard deviation of 25% (see Fig. 3, p. 23). Due to the increased variability of the results of different methods, evaluation was carried out using the z'-score, taking into account the standard uncertainty.

All data are for information only.

Important Note:
Since at least 5 results were not available for any method, a joint evaluation of the results was carried out for information purposes only. It should be noted that the resulting target range is not valid for a single ELISA method.

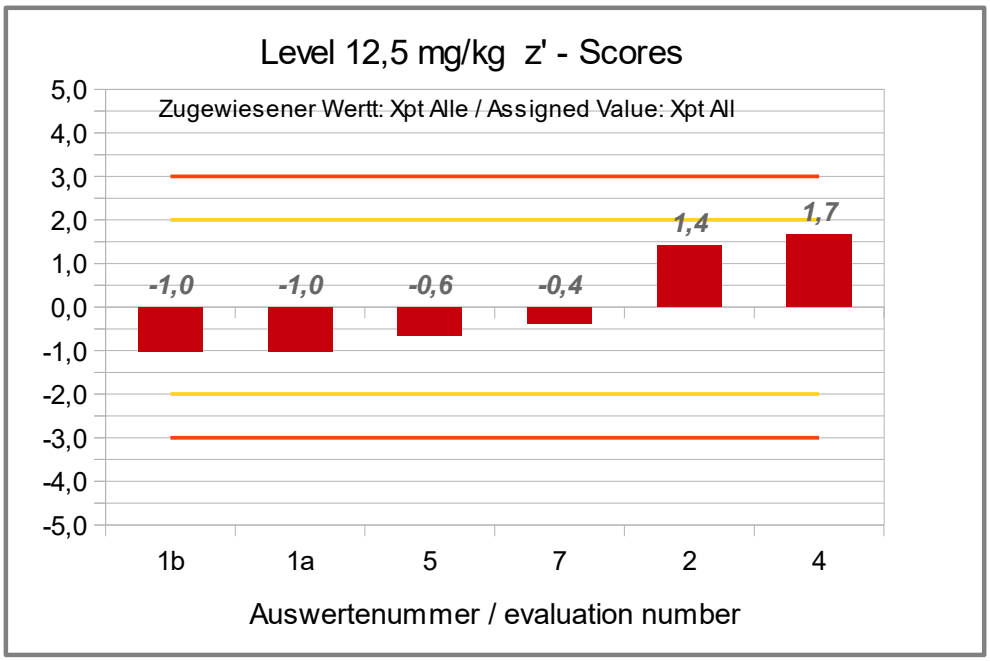


Abb./Fig. 3:
z'-Scores level 12,5 mg/kg (ELISA-results as mustard)
Assigned value: robust mean (alg. A) of all results

4.1.3.2 PCR-Methods

There were only four 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 Mustard					PCR Mustard				
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 1	Level 2	Level 3	Level 4	Level 5
1a		-1,5	-2,6	-3,1	-3,4					
1b		-1,5	-3,0	-3,1	-3,1					
2				-1,8	-2,2	-2,4	-2,4	-1,6	0,32	-2,8
3a						83	22	26	20	17
3b						51	12	9,9	5,9	8,1
4	-0,67	-2,0	-1,4	-1,7	-0,51					
5				-2,9	-3,1					
6a										
6b		2,5	3,1		-3,0					
7	-1,4	-2,8	-2,5	-2,8	-2,8	0,44	-0,10	0,0	-0,15	-0,32

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 5,0 mg/kg		Result Sample 2 Level 0,5 mg/kg		Result Sample 3 Level 25 mg/kg		Result Sample 4 „Blank“ Sample		Result Sample 5 Level 2,5 mg/kg		Result Sample 6 Level 12,5 mg/kg		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	z.B. Lebensmittel / Protein	Test-Kit + Anbieter
AQ	2	03.12.20	negative	< 2	negative	< 2	positive	11,6	negative	< 2	negative	< 2	positive	6,8	2	2		Mustard	AgraQuant ELISA Mustard COKAL2148, RomerLabs
NL	1a	09.12.20	positive	1,75	negative	0	positive	3,64	negative	0	positive	1,6	positive	2,9	1	2		Mustard	NutriLinia
RS-F	7	18.01.	positive	1,85	positive	0,327	positive	7,31	negative	< 0,5	positive	0,759	positive	3,93	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	1b	09.12.	positive	1,3	negative	0	positive	5,4	negative	0	positive	1,6	positive	2,9	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	6a	07.12.20	positive		negative	<0,50	positive		negative	<0,5	positive		positive		0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
RS-F	6b	07.01.21	positive	8,99	-		positive	6,5	-		positive	4,08	positive	>13,5	0,1	0,5		Mustard	Ridascreen® FAST Mustard R6152, R-Biopharm
SP	4	15.12.20	positive	3,27	positive	0,42	positive	21,98	negative	0,12	positive	1,26	positive	7,21	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins
SP	5	01.12.20	positive	<2	negative	<2	positive	5,6	negative	<2	negative	<2	positive	3,5	1	2		Mustard	SensiSpec ELISA Mustard, Eurofins

* NWG Nachweisgrenze / 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	2		as per kit instructions	no	
NL	1a	Mustard proteins	Allergen extraction buffer / 15 min/ 60°C	yes	Addition of skimmed milk; sample 1 and sample 5: slightly positive
RS-F	7		as per kit instructions	yes	Standard row extended by dilution of Std. 2 (0,5mg/kg)
RS-F	1b	Mustard proteins	Allergen extraction buffer / 10 min/ 60°C	yes	Addition of skimmed milk
RS-F	6a		as per kit instructions	yes	
RS-F	6b		as per kit instructions with skimmed milk powder	yes	clearly higher results with addition of 1g skimmed milk powder per sample
SP	4				addition of confidential extraction additive
SP	5	Mustard proteins	as per kit instructions	yes	

5.1.2 PCR-Methods

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 Level 5,0 mg/kg		Result Sample 2 Level 0,5 mg/kg		Result Sample 3 Level 25 mg/kg		Result Sample 4 Nullprobe		Result Sample 5 Level 2,5 mg/kg		Result Sample 6 Level 12,5 mg/kg		NWG / LOD *	BG / LOQ *	MU*	Quantitativee Result given as	Method
			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
ASU	5	02.12.20	positive		negative		positive		negative		positive		positive		2			Mustard-DNA	ASU §64 Methode/method
SFA	7	25.01.	positive	5,04	positive	0,56	positive	23,2	negative	< 1,0	positive	2,46	positive	12,1	0,4	1		Mustard	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3a		positive	37,5	positive	11	positive	133	negative	1	positive	16,5	positive	76	0,1	1		Mustard	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	3b		positive	17,5	positive	7	positive	76	negative	1	positive	10	positive	31				Mustard	Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-Q	2	30.11.20	positive	3	positive	0,2	positive	7,3	positive	0,1	positive	1	positive	13,6	0,1	4		Mustard	Sure Food Allergen Quant, R-Biopharm / Congen

* NWG Nachw eisgrenze / 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	
ASU	5		CTAB / Proteinase K / RNase A / Promega Maxw ell / Real-Time PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-65:2017-10; Sample 6: traces at limit of detection
SFA	7		DNA-Isolation by SureFood PREP Advanced, RealTime PCR as per kit instructions	yes	At several DNA isolation and measurement = high variation of results! Sample 2 and 4 show ed clear curves. Sample 4 w ith CT value above 30 .
SFA	3a				
SFA-4p	3b				
SFA-Q	2		SureFood Prep Basic	no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALM1 Sample 1

Weight whole sample	1,10	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	100	39,9
2	4,97	80	32,2
3	5,03	80	31,8
4	4,98	90	36,1
5	5,03	101	40,2
6	4,96	96	38,7
7	5,02	96	38,2
8	5,03	96	38,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	92,4	Particles
Standard deviation	8,19	Particles
χ^2 (CHI-Quadrat)	5,09	
Probability	**65**	%
Recovery rate	132	%

Normal distribution		
Number of samples	8	
Mean	36,9	mg/kg
Standard deviation	3,28	mg/kg
rel. Standard deviaton	8,87	%
Horwitz standard deviation	9,29	%
HorRat-value	**0,95**	
Recovery rate	132	%

Microtracer Homogeneity Test
DLA ptALM1 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	22,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	77	30,9
2	4,98	65	26,1
3	5,01	89	35,5
4	4,98	94	37,8
5	4,98	85	34,1
6	5,01	99	39,5
7	5,00	85	34,0
8	5,00	100	40,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	86,7	Particles
Standard deviation	11,59	Particles
χ^2 (CHI-Quadrat)	10,83	
Probability	**15**	%
Recovery rate	157	%

Normal distribution		
Number of samples	8	
Mean	34,7	mg/kg
Standard deviation	4,64	mg/kg
rel. Standard deviaton	13,4	%
Horwitz standard deviation	9,38	%
HorRat-value	**1,4**	
Recovery rate	157	%

Microtracer Homogeneity Test
DLA ptALM1 Sample 3

Weight whole sample	1,40	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	60	24,1
2	4,98	53	21,3
3	4,98	51	20,5
4	5,03	56	22,3
5	5,02	52	20,7
6	5,03	50	19,9
7	5,05	49	19,4
8	4,96	54	21,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	53,1	Particles
Standard deviation	3,76	Particles
χ^2 (CHI-Quadrat)	1,86	
Probability	**97**	%
Recovery rate	100	%

Normal distribution		
Number of samples	8	
Mean	21,2	mg/kg
Standard deviation	1,50	mg/kg
rel. Standard deviaton	7,08	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,70**	
Recovery rate	100	%

Microtracer Homogeneity Test
DLA ptALM1 Sample 5

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	70	27,7
2	4,99	74	29,7
3	4,98	78	31,3
4	5,01	68	27,1
5	5,01	70	27,9
6	4,95	72	29,1
7	4,99	72	28,9
8	5,03	68	27,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	71,5	Particles
Standard deviation	3,61	Particles
χ^2 (CHI-Quadrat)	1,28	
Probability	**99**	%
Recovery rate	132	%

Normal distribution		
Number of samples	8	
Mean	28,6	mg/kg
Standard deviation	1,44	mg/kg
rel. Standard deviaton	5,05	%
Horwitz standard deviation	9,66	%
HorRat-value	**0,52**	
Recovery rate	132	%

Microtracer Homogeneity Test
DLA ptALM1 Sample 6

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	74	29,8
2	4,98	72	28,9
3	4,97	79	31,8
4	5,03	81	32,2
5	4,98	70	28,1
6	5,03	72	28,6
7	5,04	80	31,7
8	4,97	73	29,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	75,1	Particles
Standard deviation	4,02	Particles
χ^2 (CHI-Quadrat)	1,51	
Probability	**98**	%
Recovery rate	142	%

Normal distribution		
Number of samples	8	
Mean	30,1	mg/kg
Standard deviation	1,61	mg/kg
rel. Standard deviaton	5,35	%
Horwitz standard deviation	9,59	%
HorRat-value	**0,56**	
Recovery rate	142	%

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 ptALM1 (2020)***
PT name	***ALM-Verification Mustard: 5 calibration samples containing mustard (Sinapis alba) in Spice Mixture*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Spice Mixture/ ingredients: Paprika, turmeric, pepper, onions, marjoram, caraway seeds, chillies, garlic other food additives and mustard (except "blank sample")*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 „blank sample" : 20 g*
Storage	*Samples : room temperature (long term 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative (optional: quantitative):* *Mustard / Mustard protein / DNA* *Levels (as Mustard): 0,50 / 2,5 / 5,0 / 12,5 / 25 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 January 29th 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
		USA
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany

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

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

7. Index of references

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indicum) in rice and wheat cookies and sauce powders by PCR]
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