Evaluation Report
proficiency test

DLA ptALR1 (2020)

Response PT Mustard:

5 processed Samples Mustard flour *(Sinapis alba)*, table mustard *(i.a. Sinapis alba)*, Dijon mustard *(Brassica spp),* vegetarian spread (heated) and cracker (baked)

in Potato Powder Matrix

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

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

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

2. Realisation

2.1 Test material

6 PT-samples for qualitative and optionally quantitative determination of mustard in mustard flour (*Sinapis alba)*, table mustard *(*mostly *Sinapis alba)*, Dijon mustard *(Brassica spp)*, vegetarian spread (heated) and salt cracker (baked) in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce partly processed mustard products and crackers processed by DLA. For each PT-sample 5-9 products of different origin were worked up.

Premixes with contents from approx. 0,026 - 5,0 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed, mixed gravimetrically, eventually freeze-dried (table mustard, Dijon mustard, vegetarian spread) or baked (mustard crackers), ground and homogenized. Afterwards the raw materials were mixed with further ingredients, crushed and homogenized by a ball mill.
The allergen-premixes were added to the carrier matrix of potato powder / maltodextrin (mesh < 500 µm) and homogenized. An aliquot of the carrier matrix was provided as the “blank sample”.

The 6 PT-samples were portioned to approximately 20 g in metallized PET film bags.
The contents of mustard of the PT-samples were in the range of 40 to 42 mg/kg (see Tab. 1).

Each assigned value, here the spiked allergen-contents, is afflicted with a standard uncertainty. As uncertainties the following factors were considered: protein content of spiking materials, mixing homogeneity, homogeneity and stability of mustard protein.
All uncertainties were expressed in the form of their standard deviations and then added as variances. The square root from the sum of the total variances results in the combined uncertainty “Uc”. Multiplied with the coverage factor k=2 the extended uncertainties of the assigned values "$U(X_{pt})$" are obtained [3, 13, 16-17].

Table 1: Composition of DLA-Samples

PT-Sample series	**Sample 1** Table Mustard	**Sample 2** Dijon Mustard	**Sample 3** Vegetarian Spread	**Sample 4** Salt Cracker	**Sample 5** Mustard Flour	**Sample 6** „blank"
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Potato powder Ingredients: potato, E471, E304, E223, E100 Nutrients per 100 g: Protein 8,3 g, carbohydrates 76 g, fat 0,6 g, salt 0,15 g	75	75	75	62	74	75
Maltodextrin	25	25	25	21	25	25
Allergen-Premixes Ingredients (sample 1, 2, 3 and 5): maltodextrin (94% - 98%), silicon di-oxide (<3%), processed allergen products (each 0,026% - 5% mustard)	0,084	0,083	0,41	17	0,080	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Table Mustard (freeze-dried)** Ingredients: water, mustard seeds, spirit vinegar, salt, sug-ar, spices and other ingredients Protein 19,8 % ** (9 products, Europe)	54,8	-	-	-	-	-
*Dijon Mustard (freeze-dried)** Ingredients: water, mustard seed, spirit vinegar, salt and other ingredients Protein 19,8 % ** *(5 products, France)*	-	56,7	-	-	-	-
*Veg. Spread (freeze-dried)** Ingredients: 14% table mustard, veget-able oil, water, eggs, figs, veget-ables, sugar, lemon juice, sour cream, egg yolk, flax flour, almond flour, apple syrup, sunflower seeds, herbs, turmeric, modified corn starch, potato starch, red wine, honey, coloring: carotenes and others additives Protein 5,9 % *** *(5 products, Germany)*	-	-	695	-	-	-
*Salt Crackers** *(baked 200°C, 25 min)* Ingredients: Water, wheat flour, rapeseed oil, sugar, salt, baking powder and 0,26 % mustard *(Sin-apis alba)*(#) Protein 0,069 % *** *(#9 products, Europe, Asia)*	-	-	-	176000	-	-
*Mustard Flour (Sinapis alba)** Protein 26,1 % ** *(9 products, Europe, Asia)*	-	-	-	-	39,9	-
– as Mustard	42,0	41,4	40,9	46,6	39,9	-
Extended combined uncertainty (k=2) of mustard-content (= ± 12,7 %)	± 5,33	± 5,26	± 5,19	± 5,92	± 5,07	-

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material mixtures (total nitrogen according to Kjeldahl with F=5,30 for mustard protein)
***mustard protein content calculated from mustard content according to the declaration of the products or DLA manufacture

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1 to 5 showed a probability of 98%, 75%, 81%, 95% and 81%. 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 0,55, 0,93, 1,1, 0,68 and 0,97 respectively. The results of the microtracer analysis are given in the documentation.

2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,30 - 032 (20 - 21°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 48th week of 2020. The testing method was optional. The tests should be finished at February 5th 2021 the latest.

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

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

- *The samples 1-5 are numbered in a random order. They contain Mustard flour (Sinapis alba), table mustard (mostly Sinapis alba), Dijon mustard (mostly Brassica spp), vegetarian spread (heated) and spice cracker (baked)*
- *Please give all your quantitative results as total Mustard, if possible indicate the underlying total protein content in Mustard.*
- *Possible conversion factors for processed Mustard products are queried separately in the result submission file.*

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website.
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

15 participants submitted the results in time. One participant submitted no results.

3. Evaluation

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

In the present PT five different processed products containing the allergen mustard, mustard flour *(Sinapis alba)*, table mustard (mostly *Sinapis alba)*, Dijon mustard *(Brassica spp)*, vegetarian spread (heated) and salt cracker (baked), were provided to determine the qualitative detectability and to determine the response of the used quantitative methods.

The participant results were evaluated *qualitatively* with a score from 1-5 indicating the number of successfully detected processed products.
The quantitative results were evaluated with a Recovery-Score (*RR-Score*), which indicates the number of results with a recovery rate in the range of 50 - 150% of the spiking level.

3.1 Qualitative Score

The qualitative valuation of each participant's results was performed with Scores from 1-5 considering the number of "positive" or "negative" results matching the **spiking of the PT-sample series** (see Tab. 2).
A Score from 5 indicates, that all processed products were detected successfully.
The results of the matrix sample no. 6 ("blank"-sample) were not evaluated if the participant result is in accordance with ≥75% positive or negative results of participants (consensus value) or if the result is below the limit of quantification of the used method.

Table 2: Evaluation of results using qualitative Scores

Sample 1 Table Mustard	Sample 2 Dijon Mustard	Sample 3 Vegetarian Spread	Sample 4 Salt Cracker	Sample 5 Mustard Flour	Sample 6 „blank"	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not sucessful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

3.2 Recovery-Score (RR-Score)

The evaluation of the quantitative participant results for the spiked **PT-samples** was done by recovery scores (*RR-Scores*) which are related to the number of recovery rates in the range of acceptance. The RR-Scores are calculated by counting the number of results in the range of acceptance (s. below) per number of quantitatively determined samples. Further the percentage is given in the brackets behind.

The recovery rates were calculated considering the content of the spiked allergen (level of addition). The reference values are calculated from the values for samples 1 to 5 given in section 2.1 Sample material in Table 1. As range of acceptance RA for the evaluation of the participant results the range of the AOAC-recommendation of 50-150% for allergen-ELISAs was used [21]. This range was also used in the present PT for quantitative PCR- and LC/MS-results.

Only exact quantitative results were considered. Single results outside the given measuring range (e.g. indicated with > 25 mg/kg or < 2,5 mg/kg) or indicated with "0" were not considered.

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

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 12% - 176% for PCR methods, depending on matrix or processing and concentration (s. Table 3a and 3b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 3a: ELISA-Methods – Recovery rates and precision data from selected precision experiments [30-31].

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 [24]. 12 food samples with gliadin contents in the range if 0 - 168 mg/kg were analysed by 20 laboratories. The obtained recovery rates were in the range between 65 and 110%, the relative repeatability standard deviation was between 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 [24].

The IRMM (Institute for Reference Materials and Measurements) proved the suitability of five different ELISA-Kits for the determination of peanut [27]. The mean values were in the concentration range of 0,3 - 16,1 mg/kg and/or 1,2 - 20,4 mg/kg. The smallest relative reproducibility standard deviation for each Kit was obtained for dark chocolate at 20 - 42% and cookies at 23 - 61%.

Table 3b: PCR-Methods - Recovery rates and precision data from selected precision experiments [32-36].

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**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 [25], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [22-24], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [26] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [21].

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

Table 4: ELISA validation criteria

Literature [21-26]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% (a)	19,5 - 57,2% (a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

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

Table 5: PCR validation criteria

Literature [20]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

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

3.3 z-Score (Spiking Levels)

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

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

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

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

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

3.4 z'-Score (Spiking Levels)

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered. The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [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.
Evaluation was done separately for ELISA- (and Lateral Flow) and PCR-methods.

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

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

In the present PT, all ELISA results were given as mustard or mustard flour, so that no conversions were necessary.

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

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

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

Evaluation number	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		

* Recovery Rate

4.1 Proficiency Test Processed Mustard Products

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Sample 1 Table Mustard	Sample 2 Dijon Mustard	Sample 3 Vegetarian Spread	Sample 4 Salt Cracker	Sample 5 Mustard Flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
13	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
3	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
8	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
10	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
11	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
14	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
1	positive	positive	positive	positive	positive	negative	5 (100%)	SP	
4	positive	positive	positive	positive	positive	negative	5 (100%)	SP	
5	positive	positive	positive	positive	positive	negative	5 (100%)	SP	
9	positive	positive	-	positive	positive	negative	4 (80%)	SP	
12	positive	positive	positive	positive	positive	negative	5 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	12	12	11	12	12	0
Number negative	0	0	0	0	0	12
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.1.2 Qualitative Scores: PCR-Methods

4.1.2.1 Mustard, general

Evaluation number	Sample 1 Table Mustard	Sample 2 Dijon Mustard	Sample 3 Vegetarian Spread	Sample 4 Salt Cracker	Sample 5 Mustard Flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
5	positive	positive	negative	positive	positive	negative	4 (80%)	ASU	
3	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
7	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
8	positive	positive	negative	positive	positive	negative	4 (80%)	SFA	
9	positive	positive	-	positive	positive	negative	4 (80%)	SFA	
12	positive	positive	-	positive	positive	negative	4 (80%)	SFA	
15	positive	positive	-	positive	positive	negative	4 (80%)	SFA	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	7	7	2	7	7	0
Number negative	0	0	2	0	0	7
Percent positive	100	100	50	100	100	0
Percent negative	0	0	50	0	0	100
Consensus value	positive	positive	none	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food Allergen, R-Biopharm / Congen

Comments:

For samples 1, 2, 4 and 5, consensus values of 100% positive results were obtained by PCR methods. For the processed sample 3 (spread) two positive and two negative results were obtained; another 3 participants did not submit any information for sample 3.

4.1.2.2 Mustard, yellow *(Sinapis alba)*

Evaluation number	Sample 1 Table Mustard	Sample 2 Dijon Mustard	Sample 3 Vegetarian Spread	Sample 4 Salt Cracker	Sample 5 Mustard Flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
2	positive	negative	negative	positive	positive	negative	3/3 (100%)	ASU	
5	negative	negative	negative	positive	positive	negative	2/3 (67%)	ASU	
13	positive	negative	negative	positive	positive	negative	3/3 (100%)	ASU	
6	positive	positive	-	negative	negative	-	1/3 (33%)	div	
10	positive	negative	negative	positive	positive	negative	3/3 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	4	1	0	4	4	0
Number negative	1	4	4	1	1	4
Percent positive	80	20	0	80	80	0
Percent negative	20	80	100	20	20	100
Consensus value	positive	negative	negative	positive	positive	negative
Spiking	positive	negative	-	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method

div = keine genaue Angabe / andere Methoc

div = not indicated / other method

Comments:

For all samples, consensus values of 80 or 100% positive or negative results were obtained by PCR methods. The results for samples 1, 4 and 5, which were known to contain yellow mustard (Sinapis alba), were qualitatively assessed. Sample 2 (Dijon mustard) contained Brassica species and for sample 3 (spread) the mustard species were not known.

4.1.2.3 Mustard, brown and black *(Brassica juncea / nigra)*

Evaluation number	Sample 1 Table Mustard	Sample 2 Dijon Mustard	Sample 3 Vegetarian Spread	Sample 4 Salt Cracker	Sample 5 Mustard Flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
6	positive	positive	negative	negative	negative	negative	4/4 (100%)	ASU	
7	positive	positive	negative	negative	negative	negative	4/4 (100%)	ASU	
13b	positive	positive	positive	negative	negative	negative	4/4 (100%)	ASU	
13a	positive	negative	-	positive	positive	-	3/4 (75%)	div	
12	positive	positive	positive	negative	negative	negative	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	5	4	2	1	1	0
Number negative	0	1	2	4	4	4
Percent positive	100	80	50	20	20	0
Percent negative	0	20	50	80	80	100
Consensus value	positive	positive	none	negative	negative	negative
Spiking	positive	positive	-	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

div = keine genaue Angabe / andere Methoc

div = not indicated / other method

Comments:

For samples 1, 2, 4 and 5, consensus values of 80% and 100% positive or negative results were obtained by PCR methods. For the processed sample 3 (spread) two positive and two negative results were obtained; another participant did not submit any information for sample 3.

The results for samples 1, 2, 4 and 5 for which it was known that they contain Brassica species (samples 1 and 2) or contain no Brassica species (samples 4 and 5) were evaluated qualitatively. The mustard species were not known for sample 3 (spread).

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

Evaluation number	Sample 1 Table Mustard		Sample 2 Dijon Mustard		Sample 3 Vegetarian Spread		Sample 4 Salt Cracker		Sample 5 Mustard Flour		RR- score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		
13												AQ	
3	89,8	214	101	244	64,5	158	6,44	14	49,7	**125**	1/5 (20%)	RS-F	
6	74,0	176	106	256	67,0	164	5,30	11	61,0	153	0/5 (0%)	RS-F	
8	75,6	180	112	271	77,7	190	4,65	10	70,3	176	0/5 (0%)	RS-F	
10	50,0	**119**	65,0	157	50,0	**122**	5,00	11	50,0	**125**	3/5 (60%)	RS-F	
11	49,0	**117**	83,4	201	50,2	**123**	4,90	11	44,8	**112**	3/5 (60%)	RS-F	
14	74,0	176	78,0	188	65,0	159	4,80	10	46,0	**115**	1/5 (20%)	RS-F	
1	22,5	**54**	18,2	44	18,0	44	6,50	14	72,7	182	1/5 (20%)	SP	
4	65,3	155	56,1	**135**	52,7	**129**	7,15	15	68,8	172	2/4 (50%)	SP	
5	17,0	40	25,0	**60**	24,0	**59**	5,90	13	97,0	243	2/4 (50%)	SP	
9												SP	
12	34,4	**82**	34,1	**82**	24,1	**59**	6,90	15	70,7	177	3/5 (60%)	VT	
	RA**	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**			
	Number in RA	**4**	Number in RA	**3**	Number in RA	**5**	Number in RA	**0**	Number in RA	**4**			
	Percent in RA	**40**	Percent in RA	**30**	Percent in RA	**50**	Percent in RA	**0**	Percent in RA	**40**			

Methods:
AQ = AgraQuant, RomerLabs
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

** Acceptance range of AOAC for allergen ELISAs

Comments:
For samples 1, 2, 3 and 5, 30% to 50% of the recovery rates of the participant results were in the range of the AOAC recommendation of 50-150%. For sample 4 (cracker) the recovery rates of 10-15% were well below this range.

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

4.1.4.1 Mustard, general

Evaluation number	Sample 1 Table Mustard		Sample 2 Dijon Mustard		Sample 3 Vegetarian Spread		Sample 4 Salt Cracker		Sample 5 Mustard Flour		RR- score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		
5												ASU	
3												SFA	
7	8,00	19	43,5	**105**	1,00	2,4	78,0	167	241	604	1/5 (20%)	SFA	
8	< 1		0,43	1,0	< 1		3,83	8,2	6,54	16	0/5 (0%)	SFA	
9												SFA	
12	N/A		N/A		N/A		N/A		N/A			SFA	
15	-		-		-		-		-			SFA	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	0	Number in RA	1	Number in RA	0	Number in RA	0	Number in RA	0			
	Percent in RA	0	Percent in RA	50	Percent in RA	0	Percent in RA	0	Percent in RA	0			

Methods:
ASU = ASU §64 Methode/method
SFA = Sure Food Allergen, R-Biopharm / Congen

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

** Acceptance range of AOAC for allergen ELISAs

Comments:
Two participants determined quantitative results by PCR methods. One of the recovery rates was within the range of the AOAC recommendation of 50-150%.

4.1.4.2 Mustard, yellow *(Sinapis alba)*

Evaluation number	Sample 1 Table Mustard		Sample 2 Dijon Mustard		Sample 3 Vegetarian Spread		Sample 4 Salt Cracker		Sample 5 Mustard Flour		RR- score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA **		
2	<10						15	32	53	**133**	1/2 (50%)	ASU	
5												ASU	
13												ASU	
6												div	
10	>5		<5		<5		65	**139**	65	163	1/2 (50%)	div	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	-	Number in RA	-	Number in RA	-	Number in RA	**1**	Number in RA	**1**			
	Percent in RA	-	Percent in RA	-	Percent in RA	-	Percent in RA	**50**	Percent in RA	**50**			

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

** Acceptance range of AOAC for allergen ELISAs

Methods:
ASU = ASU §64 Methode/method
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
Two participants determined quantitative results by PCR methods. Two of the recovery rates were within the range of the AOAC recommendation of 50-150%.

4.1.4.3 Mustard, brown and black *(Brassica juncea / nigra)*

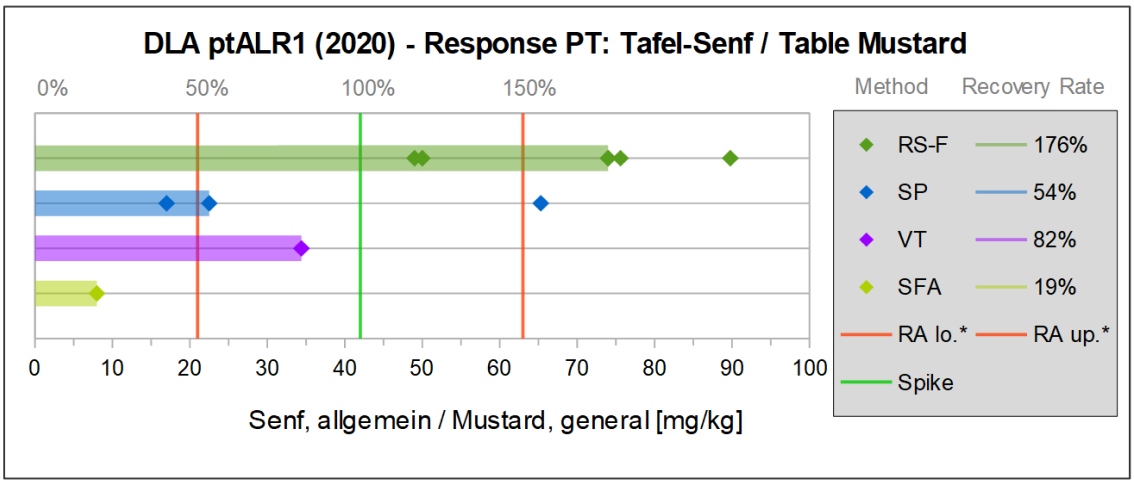
Evaluation number	Sample 1 Table Mustard		Sample 2 Dijon Mustard		Sample 3 Vegetarian Spread		Sample 4 Salt Cracker		Sample 5 Mustard Flour		RR- score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA **		
2	<5		11,0	27							0/1 (0%)	ASU	
5												ASU	
13												ASU	
6												div	
10	>1		15,0	36	>1		<1		<1		0/1 (0%)	div	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	-	Number in RA	0	Number in RA	-	Number in RA	-	Number in RA	-			
	Percent in RA	-	Percent in RA	0	Percent in RA	-	Percent in RA	-	Percent in RA	-			

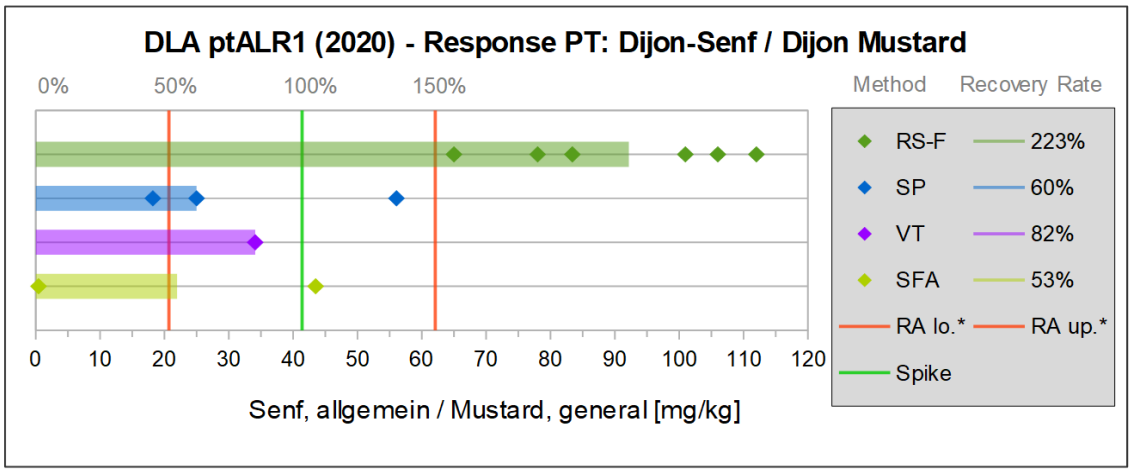
Methods:
ASU = ASU §64 Methode/method
div = keine genaue Angabe / andere Methode
div = not indicated / other method

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

** Acceptance range of AOAC for allergen ELISAs

Comments:
Two participants determined quantitative results by PCR methods. The recovery rates were below the range of the AOAC recommendation of 50-150%.





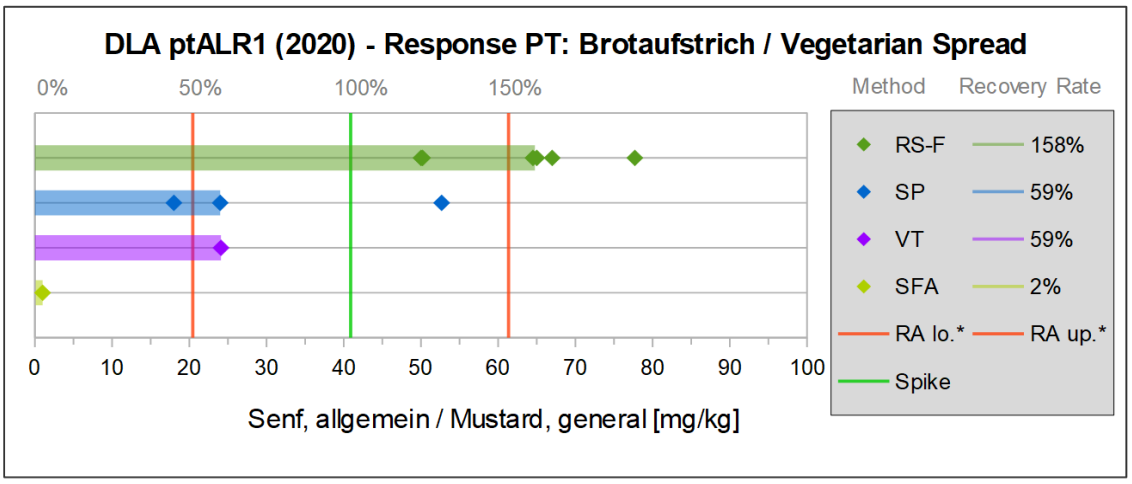
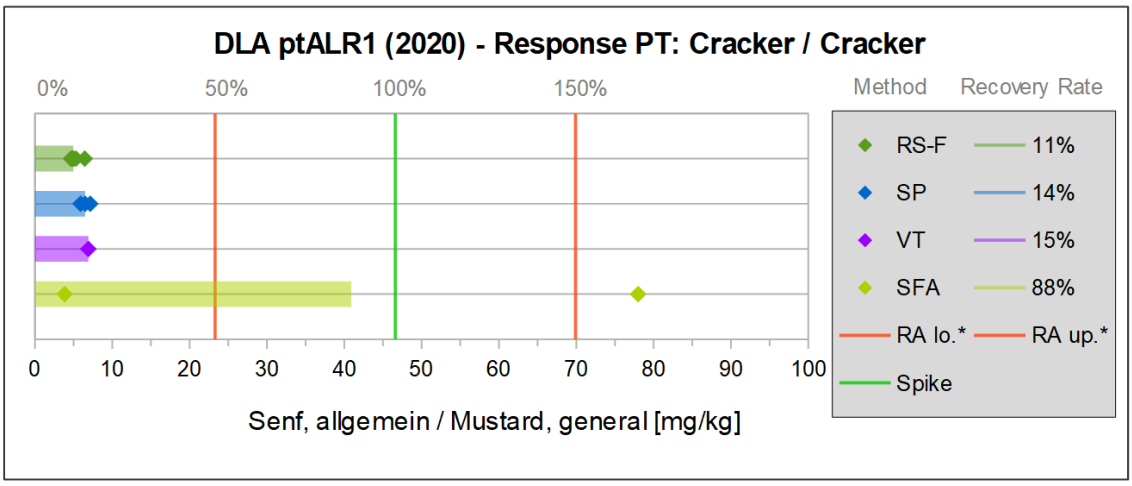


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale mustard, general, content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



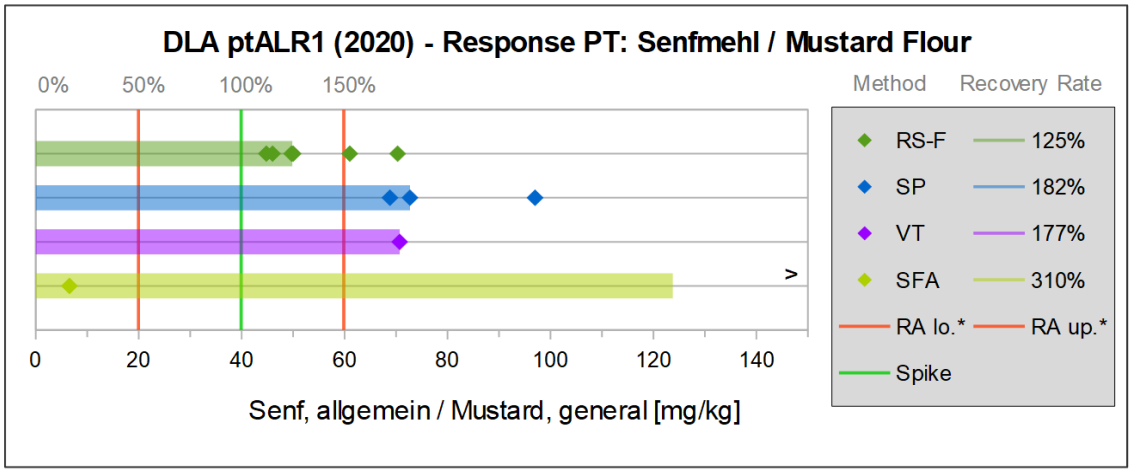


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale mustard, general, content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	preferred as Mustard
AQ	13	02.02.21	positive		positive		positive		positive		positive		negative		2			Mustard
RS-F	3	08.12.20	-	89,78	-	100,96	-	64,51	-	6,44	-	49,71	-	< BG		0,5		Mustard
RS-F	6	14.01.21	positive	74	positive	106	positive	67	positive	5,3	positive	61	negative	<0,5	0,11	0,5	0,5	Mustard
RS-F	8	05.01.	positive	75,6	positive	112	positive	77,7	positive	4,65	positive	70,3	negative	< 0,5	0,1	0,5		Mustard
RS-F	10	14.01.21	positive	50	positive	65	positive	50	positive	5	positive	50	negative	-	0,1	0,5		Mustard flour
RS-F	11	26.01.21	positive	49	positive	83,4	positive	50,2	positive	4,9	positive	44,8	negative	< 0,5	0,5			Mustard
RS-F	14	01.12.20	positive	74	positive	78	positive	65	positive	4,8	positive	46	negative	<0,5	0,11	0,5		Mustard flour
SP	1	10.12.20	positive	22,5	positive	18,2	positive	18	positive	6,5	positive	72,7	negative	<2		2		Mustard
SP	4	08.01.21	positive	65,3	positive	56,06	positive	52,68	positive	7,15	positive	68,8	negative	0	1	2		Mustard
SP	5	29.12.20	positive	17	positive	25	positive	24	positive	5,9	positive	97	negative	<2	1	2		Mustard
SP	9	15.01.21	positive		positive		-		positive		positive		negative		2	1	0,3	Please select!
VT	12	21.12.20	positive	34,4	positive	34,1	positive	24,1	positive	6,9	positive	70,7	negative	N/A	N/A	2,5	N/A	Mustard

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

Continuation details by participants: ELISA-Methods

Method Abr.	Evalu-ation number	Method	Specificity	Total protein content in sesame (According to method prescription)	Conversion for processed mustard	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	%	Recalculation from X to Y (factor or %)	e.g. Extraction solution / time / temperature	yes/no	
AQ	13	AgraQuant ELISA Mustard COKAL2148, RomerLabs					yes	results related to yellow mustard seed; cross-reactivity to black mustard 50%, to brown mustard 59%, to field mustard 48% and to rapeseed 59%
RS-F	3	Ridascreen® FAST Mustard R6152, R-Biopharm	detects specifically different species (yellow, brown, black mustard)			as per kit instructions	yes	
RS-F	6	Ridascreen® FAST Mustard R6152, R-Biopharm					no	
RS-F	8	Ridascreen® FAST Mustard R6152, R-Biopharm				as per kit instructions	yes	
RS-F	10	Ridascreen® FAST Mustard R6152, R-Biopharm	detects yellow, brown and black mustard	31.27g/100g		as per kit instructions	yes	
RS-F	11	Ridascreen® FAST Mustard R6152, R-Biopharm		31.27			yes	result as mustard flour
RS-F	14	Ridascreen® FAST Mustard R6152, R-Biopharm		31,27		AEP/10min/60°C	yes	
SP	1	SensiSpec ELISA Mustard, Eurofins					yes	
SP	4	SensiSpec ELISA Mustard, Eurofins						
SP	5	SensiSpec ELISA Mustard, Eurofins	detects mustard proteins	30-35		as per kit insert	yes	
SP	9	SensiSpec ELISA Mustard, Eurofins						
VT	12	Veratox Mustard, Neogen	unknown	0,26		as per kit insert	yes / no	

5.1.2 PCR-Methods

Mustard, general

Method Abr.	Evalu-ation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantita-tive result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as almond
ASU	5	06.01.21	positive		positive		negative		positive		positive		negative		5			Mustard-DNA
SFA	3	09.12.20	positive		positive		positive		positive		positive		negative					Mustard-DNA
SFA	7		positive	8	positive	43,5	positive	1	positive	78	positive	241	negative					Mustard
SFA	8	08.01.	positive	< 1	positive	0,43	negative	< 1	positive	3,83	positive	6,54	negative	< 1	0,4	1		Mustard
SFA	9	15.01.21	positive		positive		-		positive		positive		negative		0,4			Please select!
SFA	12	14.01.21	positive	N/A	positive	N/A	-	N/A	positive	N/A	positive	N/A	negative	N/A	0,4	1	N/A	Mustard
SFA	15	12.01.21	positive	-	positive	-	-	-	positive	-	positive	-	negative	-	0,4			Please select!

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

Mustard (Sinapis alba)

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as almond
ASU	2	21.12.20	positive	<10	negative		negative		positive	15	positive	53	negative		2	10	0,5	other: yellow mustard
ASU	5	06.01.21	negative		negative		negative		positive		positive		negative		20			DNA white mustard
ASU	13	11.01.21	positive		negative		negative		positive		positive		negative		5 pg			Mustard DNA
div	6	18.12.20	positive		positive		-		negative		negative		-		30			Mustard
div	10		positive	>5	negative	<5	negative	<5	positive	65	positive	65	negative	<5	5	25		Mustard flour

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

Mustard (Brassica ssp.)

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as almond
ASU	2	21.12.20	positiv	<5	positiv	11	negativ		negativ		negativ		negativ		1	5	0,5	other: brown mustard / black mustard
ASU	5	06.01.21	positiv		positiv		negativ		negativ		negativ		negativ		5			DNA brown/black mustard
ASU	13	11.01.21	positiv		positiv		positiv		negativ		negativ		negativ		1 pg			Mustard DNA
div	6	18.12.20	positive		negative		-		positive		positive		-		5			Mustard
div	10		positiv	>1	positiv	15	positiv	>1	negativ	<1	negativ	<1	negativ	<1	1	10		mustard flour

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

Continuation details by participants: PCR-Methods

Mustard, general

Method Abr.	Evalu-ation Number	Method	Specificity	Total protein content in sesame (According to method prescription)	Conversion for processed sesame	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	%	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
ASU	5	ASU §64 Methode/method				CTAB / Proteinase K / RNase A / Promega Maxw ell / Real-Time PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-65:2017-10
SFA	3	Sure Food ALLERGEN, R-Biopharm / Congen	characteristic sequence part of mustard DNA			SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
SFA	7	Sure Food ALLERGEN, R-Biopharm / Congen				CTAB-Extraction/ Clean-up w ith column (QIAquick) Real-Time PCR according to manual	yes	
SFA	8	Sure Food ALLERGEN, R-Biopharm / Congen				DNA Isolation by SureFood PREP Advanced, Real Time PCR according to manual	yes	Sample 1: approx. 0,26mg/kg; Sample 2: > LOD and < LOQ ; Sample 3: clear curve, not quantifiable, result "traces"; Sample 6: no noise signal
SFA	9	Sure Food ALLERGEN, R-Biopharm / Congen						sample 1 w eakly positive
SFA	12	Sure Food ALLERGEN, R-Biopharm / Congen				SureFood Prep Advanced art no S1053, protocole 2	no	
SFA	15	Sure Food Allergen ID, R-Biopharm / Congen					Yes	

Mustard *(Sinapis alba)*

Method Abr.	Evalu-ation Number	Method	Specificity	Total protein content in mustard (According to method prescription)	Conversion for processed mustard	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	%	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
ASU	2	ASU §64 Methode/method	mRNA of MADS-D-Protein from Sinapis alba	not applicable	not applicable	ASU L 08.00-65 (Multiplex PCR; semi-quant. screening); Quantification according to ASU L08.00-59	yes	
ASU	5	ASU §64 Methode/method				CTAB / Proteinas K / RNase A / Promega Maxw ell / Real-Time PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-65:2017-10
ASU	13	ASU §64 Methode/method				DNeasy mericon Food Kit (Qiagen)	yes	
div	6	in house method					yes	
div	10	Selection PCR-Methods	MADS D			CTAB-Wizard	yes	

Mustard (Brassica ssp.)

Method Abr.	Evalu-ation Number	Method	Specificity	Total protein content in mustard (According to method prescription)	Conversion for processed mustard	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	%	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
ASU	2	ASU §64 Methode/method	Brow n and black mustard: reverse Transkriptase from Gypsy-like Retro element 13G42-26	not applicable	not applicable	ASU L 08.00-65 (Multiplex PCR; semi-quant. screening); Quantification according to ASU L08.00-59	yes	Method detects only brow n and black mustard
ASU	5	ASU §64 Methode/method				CTAB / Proteinas K / RNase A / Promega Maxw ell / Real-Time PCR / 45 Cycles	yes	§ 64 LFGB L 08.00-65:2017-10
ASU	13	ASU §64 Methode/method				DNeasy mericon Food Kit (Qiagen)	yes	
div	6	in house method					yes	
div	10	Selection PCR-Methods	gypsy-like Retroelement 13G42-26			CTAB-Wizard	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALR1 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	75	30,0
2	5,00	75	30,0
3	4,97	74	29,8
4	5,01	70	27,9
5	5,03	75	29,8
6	5,02	77	30,7
7	4,98	66	26,5
8	5,03	79	31,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	73,9	Particles
Standard deviation	3,91	Particles
χ^2 (CHI-Quadrat)	1,45	
Probability	**98**	%
Recovery rate	88	%

Normal distribution		
Number of samples	8	
Mean	29,5	mg/kg
Standard deviation	1,56	mg/kg
rel. Standard deviaton	5,30	%
Horwitz standard deviation	9,61	%
HorRat-value	**0,55**	
Recovery rate	88	%

Microtracer Homogeneity Test
DLA ptALR1 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	33,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	77	30,9
2	5,01	80	31,9
3	5,00	68	27,2
4	5,01	76	30,3
5	5,02	84	33,5
6	5,03	69	27,4
7	4,99	72	28,9
8	5,01	87	34,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	76,6	Particles
Standard deviation	6,81	Particles
χ^2 (CHI-Quadrat)	4,24	
Probability	**75**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	30,6	mg/kg
Standard deviation	2,72	mg/kg
rel. Standard deviaton	8,89	%
Horwitz standard deviation	9,56	%
HorRat-value	**0,93**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA ptALR1 Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	39	15,6
2	5,02	46	18,3
3	4,98	39	15,7
4	5,02	52	20,7
5	5,01	42	16,8
6	4,98	50	20,1
7	5,01	49	19,6
8	5,01	44	17,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,1	Particles
Standard deviation	4,91	Particles
χ^2 (CHI-Quadrat)	3,75	
Probability	**81**	%
Recovery rate	81	%

Normal distribution		
Number of samples	8	
Mean	18,0	mg/kg
Standard deviation	1,96	mg/kg
rel. Standard deviaton	10,9	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,1**	
Recovery rate	81	%

Microtracer Homogeneity Test
DLA ptALR1 Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	30,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	73	29,2
2	4,99	62	24,8
3	4,98	71	28,5
4	5,02	76	30,3
5	5,00	72	28,8
6	5,00	64	25,6
7	4,98	68	27,3
8	4,98	68	27,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,2	Particles
Standard deviation	4,59	Particles
χ^2 (CHI-Quadrat)	2,13	
Probability	**95**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	27,7	mg/kg
Standard deviation	1,84	mg/kg
rel. Standard deviaton	6,62	%
Horwitz standard deviation	9,70	%
HorRat-value	**0,68**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA ptALR1 Sample 5

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	54	21,6
2	5,03	52	20,7
3	5,02	55	21,9
4	5,01	70	27,9
5	5,00	55	22,0
6	5,02	58	23,1
7	4,99	57	22,8
8	5,00	56	22,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	57,1	Particles
Standard deviation	5,52	Particles
χ^2 (CHI-Quadrat)	3,73	
Probability	**81**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	22,8	mg/kg
Standard deviation	2,20	mg/kg
rel. Standard deviaton	9,66	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,97**	
Recovery rate	103	%

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 ptALR1 (2020)***
PT name	***Response PT Mustard: Processed samples Mustard flour (Sinapis alba), table mustard (Sinapis alba), Dijon mustard (Brassica spp), vegetarian spread (heated) and spice cracker (baked) in potato powder matrix*** *(levels: 25 - 150 mg/kg)*
*Sample matrix**	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 "Blank" Sample: 20 g*
Storage	*Samples 1-6: room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Mustard / Mustard Protein / DNA from Mustard of* ***Sinapis alba and/or Brassica spp.*** *Samples 1-5: approx. 25 - 150 mg/kg (as total Mustard)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples 1 - 6 and the* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of significant digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest 05th February 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SWITZERLAND
		Germany
		Germany
		USA
		CANADA
		Germany
		Germany
		Germany
		Germany
		Germany
		ITALY
		BRASIL
		GREAT BRITAIN
		FINLAND
		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.]

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