Evaluation Report
proficiency test

DLA ptALM2

ALM-Verification:

Sesame (white) in Spice Cracker Matrix

5 Samples baked with Sesame
(levels: 1,0 / 5 / 10 / 20 / 50 mg/kg)

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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.

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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 cracker were provided for qualitative detection and optional quantitative determination of sesame. The sesame levels of the PT-sample series were in the range from 1,0 mg/kg to 50 mg/kg, whereas the medial level represents the "Action Level" (see Table 1).
The food matrix of sample material was spice cracker. The basic composition was identical for all 6 samples (see Table 1).
After crushing and sieving using an impact mill (mesh 1,5 mm) the basic mixture was homogenized and an aliquot was taken from it as blank sample.

For preparation of the sesame containing samples first crackers were baked (200°C, 20-30 min) and dried (40°C) using a white sesame mixture (further information see below). Afterwards the sesame crackers 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 sesame containing crackers was added to the basic mixture. The resulting mixture was homogenized again. Afterwards basic mixture was added stepwise (4-5 steps) including homogenization after each step until the total amount of sample material was reached.

For the spiking a mixture of white sesame seeds from a total of 10 products (from Asia, South America and Africa) was used. For the samples (matrix: potato powder) of proficiency test DLA ptAL04 (2020) this mixture gave a mean recovery rate for sesame of 87 % ± 45 % (n=11) calculated from different ELISA methods* and of 299 % ± 28 % (n=17) calculated from the ELISA method RS-F**.

* div. ELISA methods = AgraQuant, BioCheck, BioFront Technologies, ELISA Systems (new), ELISA Systems, nutriLinia®, SensiSpec
** ELISA method RS-F = R-Biopharm, Ridascreen® Fast

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „blank“	**Level 1** 1,0 mg/kg	**Level 2** 5 mg/kg	**Level 3** 10 mg/kg	**Level 4** 25 mg/kg	**Level 5** 50 mg/kg
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Spice Crackers Ingredients: wheat flour, sunflower oil, potato flour, potato starch, modified corn starch, glucose syrup, dried tomatoes 3.9%, sea salt 2.1%, flavour, salt, oregano 0.3%, raising agent ammonium hydrogen carbonate Nutrients per 100 g: Fat 13 g, carbohydrates 70 g, protein 6,4 g	100	>99,9	99,8	99,6	99,0	98,0
Crackers (baked 200°C, 25 min) Ingredients: wheat flour, rapeseed oil, sugar, salt, baking powder and a mixture of white sesame seeds and other ingredients (maltodextrin, silicon dioxide)	–	0,040	0,20	0,40	0,99	1,98
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof Sesame: – as Sesame* – *with 20,8% protein***	–	**1,02** 0,212	**5,08** 1,06	**10,2** 2,12	**25,4** 5,28	**50,8** 10,6
Extended combined uncertainty (k=2) of sesame content (= ± 12,5 %)	± 0,13	± 0,64	± 1,3	± 3,2	± 6,4	± 0,13

*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 sesame 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 sesame.
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 (Morinaga Sesam ELISA Kit II). The spiking levels correlated with the ascending values of measured results (see Fig. 1). The recovery rates ranged from 95% to 113%. The relative standard deviation (RSD) of the action level (level 3) was approx. 113%.

Table 2: Characterization of PT-sample series sesame in spice crackers by ELISA determination (Morinaga ELISA Kit II*).
* The analysed sesameprotein results were converted with a content of 20,8 % to total sesame (see p. 6).

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	1,0	5,1	10,2	25,4	50,8
Result 1	< 0,75	0,97	5,74	10,8	27,3	51,5
Result 2	-	-	-	12,6	-	-
Result 3	-	-	-	9,69	-	-
Mean		0,97	5,74	11,0	27,3	51,5
SD	-			1,46		
RSD [%]	-			13,2		
Recovery [%]	-	95	113	108	107	101

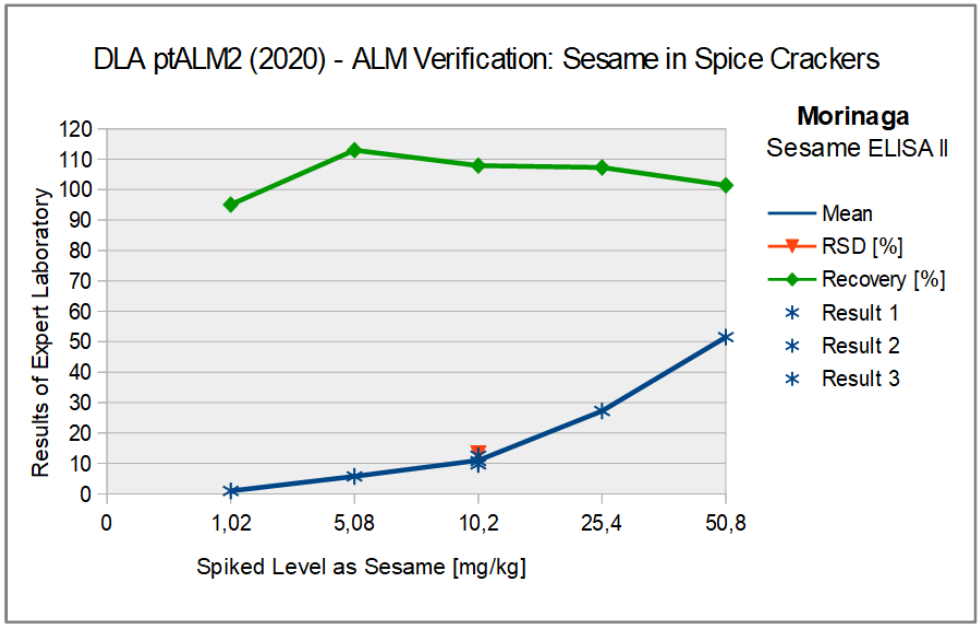


Abb./Fig. 1: ELISA results of PT-sample series sesame in spice crackers (Morinaga ELISA Kit II), *Note: the x-scale is not shown linear to obtain a better recognizability of low values.*

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 2 to 6 showed a probability of 84%, 97%, 79%, 100% and 48%. 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 0,90, 0,64, 0,92, 0,38 and 0,99 respectively. The value of 1,6 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. 0,17 (18,6°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test material (sample 1 to 6) were sent to every participating laboratory in the 18nd week of 2020. The testing method was optional. The tests should be finished at July 10th 2020 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 Sesame as well as a „blank sample" in the matrix Spice Cracker.

- *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 10 mg/kg Sesame 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 and, if possible, evaluation by z-scores.

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

2.3 Submission of results

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

All 8 participants submitted 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 1,0 mg/kg	Level 2 5 mg/kg	Level 3 (Action Level) 10 mg/kg	Level 4 25 mg/kg	Level 5 50 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 59 – 98% for PCR methods (sesame), 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]**	**Recovery**	**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 ($U_{(X_{pt})}$) [3].

The calculation is performed by:

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

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

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

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
The **qualitative and quantitative evaluations** were done **separately** for ELISA and PCR methods. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

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

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

ELISA results given as **sesame protein** were converted by DLA to **total food items (sesame seed)** using the analyzed protein content of the raw materials (see page 6).

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

Participant	Level 0 „blank“	Level 1 1,0 mg/kg	Level 2 5 mg/kg	Level 3 (Action Level) 10 mg/kg	Level 4 25 mg/kg	Level 5 50 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 – 1,0 mg/kg			Level 2 – 5 mg/kg			Level 3 – 10 mg/kg (Action Level)			Level 4 – 25 mg/kg			Level 5 – 50 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 Sesame

4.1.1 Qualitativ: Action Level Matrix-Scores

4.1.1.1 ELISA-Methods

Evaluation number	Level 0 „Null“	Level 1 1,0 mg/kg	Level 2 5 mg/kg	Level 3 (Action Level) 10 mg/kg	Level 4 25 mg/kg	Level 5 50 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		
8a	negative	positive	positive	positive	positive	positive	5 (100%)	BF	
8b	negative	negative	positive	positive	positive	positive	4 (80%)	BF-LF	Lateral Flow
5	negative	negative	positive	positive	positive	positive	4 (80%)	IL	
7	negative	negative	negative	positive	positive	positive	3 (60%)	IL	
1	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
4	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	Level 2 (Sample 3) positive < Action Level
6	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
2	negative	positive	positive	positive	positive	positive	5 (100%)	SP	
3	negative	negative	positive	positive	positive	positive	4 (80%)	SP	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	2	8	9	9	9
Number negative	9	7	1	0	0	0
Percent positive	0	22	89	100	100	100
Percent negative	100	78	11	0	0	0
Consensus value	negative	negative	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace (Lateral Flow), BioFront Technologies

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The Action Level (10 mg/kg) as well as the higher levels 4 and 5 were successfully detected by all participants. Level 2 was detected by 89% (9) of the participants, while level 1 was detected by only 22% (2). According to the test kit instructions the content of level 1 (1 mg/kg) lies between the limit of detection and the limit of quantitation *of the ELISA methods used.*

4.1.1.2 PCR-Methods

Evaluation number	Level 0 „Null“	Level 1 1,0 mg/kg	Level 2 5 mg/kg	Level 3 (Action Level) 10 mg/kg	Level 4 25 mg/kg	Level 5 50 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		
1	negative	negative	positive	positive	positive	positive	4 (80%)	ASU	
3	negative	negative	negative	positive	positive	positive	3 (60%)	ASU	
4	negative	positive	positive	positive	positive	positive	0 (0%)	SFA	All levels positive < action level
5	negative	negative	positive	positive	positive	positive	4 (80%)	SFA	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	1	3	4	4	4
Number negative	4	3	1	0	0	0
Percent positive	0	25	75	100	100	100
Percent negative	100	75	25	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

Comments:

Four participants detected the action level of 10 mg/kg and the higher levels 4 and 5. Level 2 was detected by three of the participants. Level 1 was detected by one of the participants.
According to the test kit instructions the content of level 1 (1 mg/kg) is at the limit of quantitation of the PCR method SFA.

4.1.2 Quantitative: Recovery Scores and z-Scores

4.1.2.1 ELISA-Results

Evaluation number	Level 1 – 1,0 mg/kg			Level 2 – 5 mg/kg			Level 3 – 10 mg/kg (Action Level)			Level 4 – 25 mg/kg			Level 5 – 50 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 **		
8a	0,700	**69**	-1,2	3,70	**73**	-1,1	6,20	**61**	-1,6	16,5	**65**	-1,4	26,0	**51**	-2,0	5/5 (100%)	BF	
8b																	BF-LF	Lateral Flow
5	<LOQ			2,63	**52**	-1,9	4,84	48	-2,1	10,3	40	-2,4	14,3	28	-2,9	1/4 (25%)	IL	
7							13,6	**134**	1,4	36,1	**142**	1,7	70,8	**139**	1,6	3/3 (100%)	IL	Protein-Results converted ° Mean calculated by DLA
1				6,00	**118**	0,72	10,0	**98**	-0,07	28,0	**110**	0,41	60,0	**118**	0,73	4/4 (100%)	RS-F	
4	0,870	**86**	-0,58	8,28	163	2,5	15,8	155	2,2	37,1	**146**	1,8	85,5	168	2,7	2/5 (40%)	RS-F	
6	<2,5			7,10	**140**	1,6	14,2	**140**	1,6	34,3	**135**	1,4	65,9	**130**	1,2	4/4 (100%)	RS-F	
2	0,580	**57**	-1,7	2,90	**57**	-1,7	5,50	**54**	-1,8	16,0	**63**	-1,5	20,0	39	-2,4	4/5 (80%)	SP	
3	<2			2,60	**51**	-2,0	5,40	**53**	-1,9	9,30	37	-2,5	14,0	28	-2,9	2/4 (50%)	SP	

° calculation see p. 16

RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %
Number in RA	**3**	Number in RA	**6**	Number in RA	**6**	Number in RA	**6**	Number in RA	**4**
Percent in RA	**100**	Percent in RA	**86**	Percent in RA	**75**	Percent in RA	**75**	Percent in RA	**50**

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

** Acceptance range of AOAC for allergen ELISAs

Methods:

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace (Lateral Flow), BioFront Technologies

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

For the levels 1 to 5 50% to 100% of the recovery rates of the participants' results were within the AOAC recommendations of 50-150%. The recovery rates of method RS-F were in the upper range (98-168%), while the recovery rates of the other methods were in the lower range (28-73%) (exception result no. 7).

4.1.2.2 PCR-Results

Evaluation number	Level 1 – 1,0 mg/kg			Level 2 – 5 mg/kg			Level 3 – 10 mg/kg (Action Level)			Level 4 – 25 mg/kg			Level 5 – 50 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 **		
1																	ASU	
3																	ASU	
4	0,11	10	-3,6	0,395	7,8	-3,7	0,940	9,2	-3,6	2,05	8,1	-3,7	4,42	8,7	-3,7	0/5 (0%)	SFA	Angegeben als „Sesam-DNA“
5	<0,4			0,230	4,5	-3,8	0,550	5,4	-3,8	4,89	19	-3,2	10,7	21	-3,2	0/4 (0%)	SFA	
	RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %		RA**	50-150 %				
	Number in RA	0		Number in RA	0		Number in RA	0		Number in RA	0		Number in RA	0				
	Percent in RA	0		Percent in RA	0		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

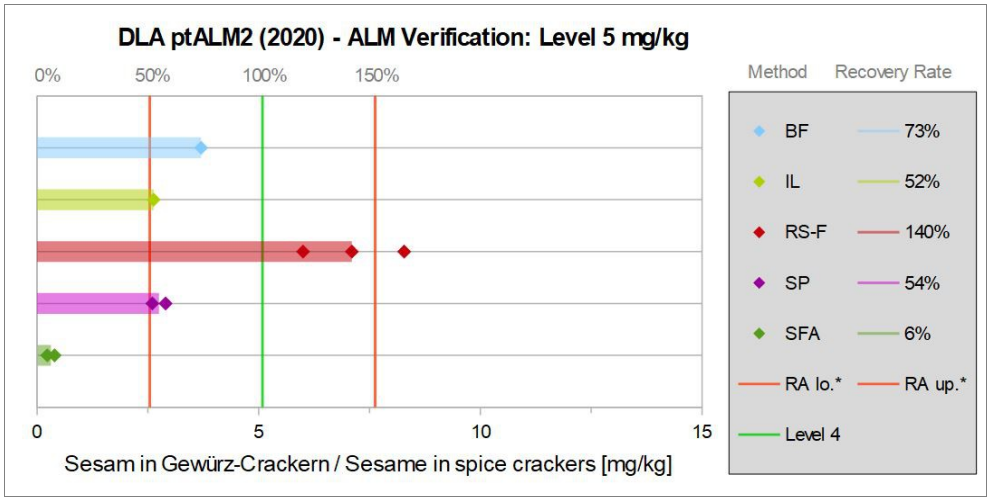
* Recovery rate 100% Reference value: Sesame, s. Page 6

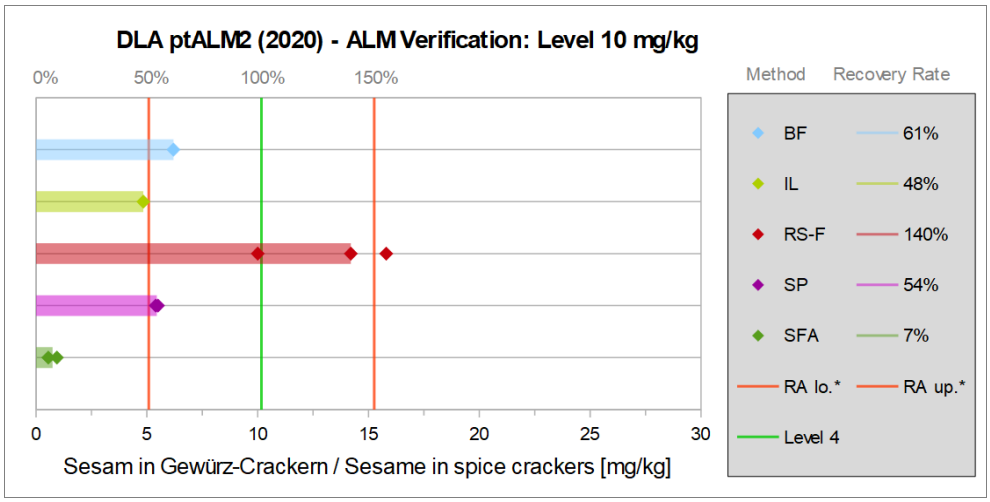
** Acceptance range of AOAC for allergen ELISAs

Comments:

Two participants reported quantitative PCR results. The recovery rates for all levels were between 5% and 21% and thus below the range of the AOAC recommendations of 50-150%.

Note: The information from participant 4 was submitted as sesame DNA, but the recovery rates calculated by DLA refer to sesame.





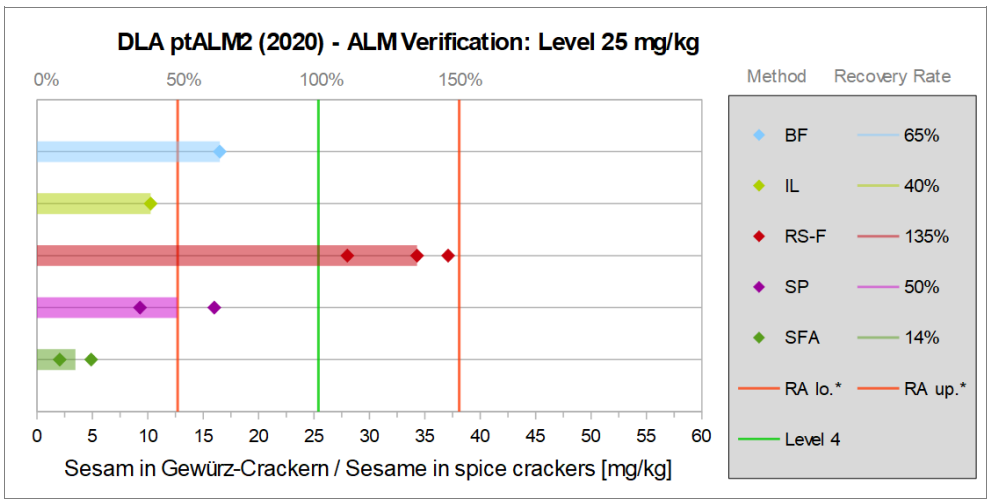


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale sesame content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)
Note: Figures without converted results no. 7, which were submitted as sesame protein.

4.1.3 Informative Data: Statistical characteristics sesame

4.1.3.1 ELISA-Methods

Sample: Action Level 10,0 mg/kg

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	8
Number of outliers	0
Mean	9,45
Median	8,10
Robust Mean (X_{pt})	**9,45**
Robust standard deviation (S*)	**5,15**
Target range:	
Target standard deviation $\sigma_{pt'}$	**3,28**
lower limit of target range	**2,89**
upper limit of target range	**16,0**
Quotient S/$\sigma_{pt'}$*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*2,28*
Results in the target range	*8*
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:
The kernel density estimate shows a bimodal distribution of the results. The results of the RS-F method gave higher values than those of the other methods (BF, IL, SP).
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.

Sample: Level 25 mg/kg

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	8
Number of outliers	0
Mean	23,4
Median	22,3
Robust Mean (X_{pt})	**23,4**
Robust standard deviation (S*)	**13,3**
Target range:	
Target standard deviation $\sigma_{pt'}$	**8,30**
lower limit of target range	**6,85**
upper limit of target range	**40,0**
Quotient S/$\sigma_{pt'}$*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*5,87*
Results in the target range	*8*
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. 4, p. 24). 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:
The kernel density estimate shows a bimodal distribution of the results. The results of the RS-F method gave higher values than those of the other methods (BF, IL, SP).
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.

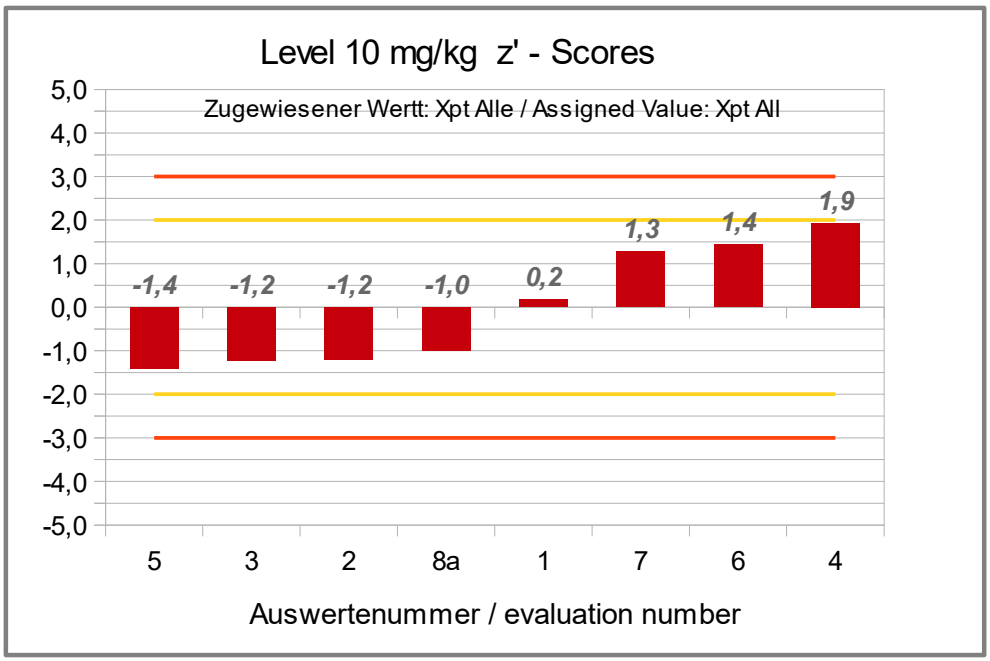


Abb./Fig. 3:
z'-Scores action level 10,0 mg/kg (ELISA-results as sesame)
Assigned value: robust mean (alg. A) of all results

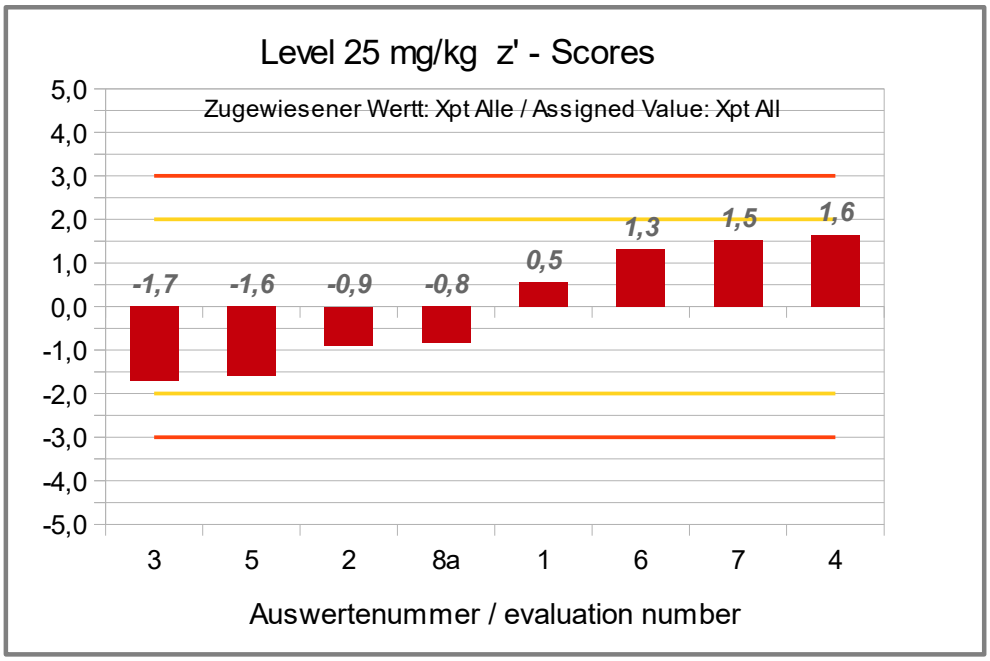


Abb./Fig. 4:
z'-Scores level 25 mg/kg (ELISA-results as sesame)
Assigned value: robust mean (alg. A) of all results

4.1.3.2 PCR-Methods

There were only two 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 Sesame					PCR Sesame				
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 1	Level 2	Level 3	Level 4	Level 5
1		0,72	-0,07	0,41	0,73					
2	-1,7	-1,7	-1,8	-1,5	-2,4					
3		-2,0	-1,9	-2,5	-2,9					
4	-0,58	2,5	2,2	1,8	2,7	-3,6	-3,7	-3,6	-3,7	-3,7
5		-1,9	-2,1	-2,4	-2,9		-3,8	-3,8	-3,2	-3,2
6		1,6	1,6	1,4	1,2					
7			1,4	1,7	1,6					
8 / 8a	-1,2	-1,1	-1,6	-1,4	-2,0					
8b										

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 Nullprobe		Result Sample 2 Level 50 mg/kg		Result Sample 3 Level 5 mg/kg		Result Sample 4 Level 10 mg/kg		Result Sample 5 Level 1 mg/kg		Result Sample 6 Level 25 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	e.g. Food / Protein	Test-Kit + Provider
BF	8a	10.07.20	negative	0	positive	26	positive	3,7	positive	6,2	positive	0,7	positive	16,5	0,16	1		Sesame	MonoTrace Sesame ELISA kit, BioFront Technologies
BF-LF	8b	10.07.20	negative		positive		positive		positive		negative		positive		2	-		Sesame	AllerTrace Sesame - BioFront Technologies
IL	5	03.07.20	negative	<LOQ	positive	14,29	positive	2,63	positive	4,84	negative	<LOQ	positive	10,26	0,5	2		Sesame	Immunolab Sesame ELISA
IL	7	03.06.20, 01.07.20	negative		positive	14,7233	negative		positive	2,83667	negative		positive	7,5		2		Sesameprotein	Immunolab Sesame ELISA
RS-F	1		negative		positive	60	positive	6	positive	10	negative		positive	28	2,5	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	4	16.06.	negative	< 2,5	positive	85,5	negative	8,28	positive	15,8	negative	0,87	positive	37,1	0,14	2,5 / 0,25		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
RS-F	6	13./ 18.05.20., 01.07.20.	negative	<2.5	positive	65,9	positive	7,1	positive	14,2	negative	<2.5	positive	34,3	2,5	2,5		Sesame	Ridascreen® FAST Sesame R7202, R-Biopharm
SP	2	05.05.20	negative	0	positive	20	positive	2,9	positive	5,5	positive	0,58	positive	16				Sesame	SensiSpec ELISA Sesame, Eurofins
SP	3	12.05.20	negative	<2	positive	14	positive	2,6	positive	5,4	negative	<2	positive	9,3	1,5	2		Sesame	SensiSpec ELISA Sesame, 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	
BF	8a	Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C		
BF-LF	8b	Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature		
IL	5			no	Sample 5 = 0,63 ppm
IL	7		Immunolab test instruction version: february 21st 2019	no	
RS-F	1			yes	
RS-F	4		as per test kit instructions	no	Standard 2,5 further diluted to 0,25mg/kg, thus LOQ is 2,5 / 0,25! As valuation for a customer sample 3 will be indicated as positive. Here we evaluated according to Action Level Vital concept!
RS-F	6		Sesame extraction Buffer containing skim milk powder/10 minutes/60°C) - extraction	yes	
SP	2				
SP	3	detects sesame proteins	as per test kit instructions	yes	

5.1.2 PCR-Methods

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 Nullprobe		Result Sample 2 Level 50 mg/kg		Result Sample 3 Level 5 mg/kg		Result Sample 4 Level 10 mg/kg		Result Sample 5 Level 1 mg/kg		Result Sample 6 Level 25 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	e.g. Food / Protein	Test-Kit + Provider
ASU	1		negative		positive		positive		positive		negative		positive					Sesame-DNA	ASU §64 Methode/method
ASU	3	13.05.20	negative		positive		negative		positive		negative		positive		20			Sesame-DNA	ASU §64 Methode/method
SFA	4	04.06.	negative	< 1	negative	4,42	negative	0,395	negative	0,94	negative	0,105	negative	2,05		1		Sesame-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	10.07.20	negative	<0,4	positive	10,72	positive	0,23	positive	0,55	negative	<0,4	positive	4,89	0,4	1		Sesame	Sure Food Allergen ID, 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	1		Extraction: M&N		currently in validation progress
ASU	3		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-Time PCR / 45 cycles	yes	
SFA	4		as per test kit instructions, SureFood PREP Advanced	yes	As valuation for a customer samples 2-6 will be indicated as positive. Here we evaluated according to Action Level Vital concept!
SFA	5			no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALM2 Sample 2

Weight whole sample	1,90	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	65	25,9
2	5,00	56	22,4
3	5,03	62	24,7
4	5,03	73	29,0
5	5,01	55	22,0
6	5,00	61	24,4
7	4,99	61	24,4
8	5,03	60	23,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	61,6	Particles
Standard deviation	5,50	Particles
χ^2 (CHI-Quadrat)	3,44	
Probability	**84**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	24,6	mg/kg
Standard deviation	2,19	mg/kg
rel. Standard deviaton	8,93	%
Horwitz standard deviation	9,88	%
HorRat-value	**0,90**	
Recovery rate	103	%

Microtracer Homogeneity Test
DLA ptALM2 Sample 3

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	64	25,5
2	4,97	64	25,8
3	5,04	70	27,8
4	4,97	63	25,4
5	4,99	72	28,9
6	4,95	61	24,6
7	4,98	70	28,1
8	5,03	72	28,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	67,0	Particles
Standard deviation	4,18	Particles
χ^2 (CHI-Quadrat)	1,83	
Probability	**97**	%
Recovery rate	100	%

Normal distribution		
Number of samples	8	
Mean	26,8	mg/kg
Standard deviation	1,67	mg/kg
rel. Standard deviaton	6,24	%
Horwitz standard deviation	9,75	%
HorRat-value	**0,64**	
Recovery rate	100	%

Microtracer Homogeneity Test
DLA ptALM2 Sample 4

Weight whole sample	0,85	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	81	32,5
2	5,03	69	27,4
3	5,01	70	27,9
4	5,00	60	24,0
5	5,02	74	29,5
6	5,02	70	27,9
7	5,03	67	26,6
8	5,03	66	26,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,6	Particles
Standard deviation	6,21	Particles
χ^2 (CHI-Quadrat)	3,88	
Probability	**79**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	27,8	mg/kg
Standard deviation	2,48	mg/kg
rel. Standard deviaton	8,92	%
Horwitz standard deviation	9,70	%
HorRat-value	**0,92**	
Recovery rate	104	%

Microtracer Homogeneity Test
DLA ptALM2 Sample 5

Weight whole sample	0,85	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	25,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	79	31,4
2	5,03	81	32,2
3	4,98	80	32,1
4	5,03	76	30,2
5	5,04	76	30,2
6	4,98	79	31,7
7	5,01	79	31,5
8	5,03	73	29,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	77,9	Particles
Standard deviation	2,83	Particles
χ^2 (CHI-Quadrat)	0,72	
Probability	**100**	%
Recovery rate	120	%

Normal distribution		
Number of samples	8	
Mean	31,1	mg/kg
Standard deviation	1,13	mg/kg
rel. Standard deviaton	3,63	%
Horwitz standard deviation	9,54	%
HorRat-value	**0,38**	
Recovery rate	120	%

Microtracer Homogeneity Test
DLA ptALM2 Sample 6

Weight whole sample	0,85	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	132	52,6
2	5,01	105	41,9
3	5,03	126	50,1
4	5,00	126	50,4
5	5,03	104	41,4
6	5,02	114	45,4
7	4,99	126	50,5
8	4,98	119	47,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	119,0	Particles
Standard deviation	10,52	Particles
χ^2 (CHI-Quadrat)	6,51	
Probability	**48**	%
Recovery rate	143	%

Normal distribution		
Number of samples	8	
Mean	47,5	mg/kg
Standard deviation	4,20	mg/kg
rel. Standard deviaton	8,84	%
Horwitz standard deviation	8,95	%
HorRat-value	**0,99**	
Recovery rate	143	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptALM2 (2020)***
PT name	***ALM-Verification Sesame: 5 samples containing Sesame in Spice Cracker Matrix*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Spice Crackers (baked at appr. 200°C)/ ingredients: wheat flour, sunflower oil, potato flour, potato starch, modified corn starch, glucose syrup, dried tomatoes 4%, salt 2.3%, aroma, oregano, raising agents as well as water, rapeseed oil, sugar, other food additives and sesame (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):* *Sesame / Sesame protein / DNA* *Levels (as Sesame): 1,0 / 5,0 / 10 / 25 / 50 mg/kg*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably the total sample amount should be homogenized.*
Result sheet	*One qualitative (and optional quantitative) result each should be determined for Samples 1-6.* *The results should be filled in the result submission file.*
Units	*positive / negative (optional: mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest July 10th 2020***
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
		PORTUGAL
		Germany
		Germany
		SCOTLAND
		GREECE

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. EN ISO/IEC 17034:2016; Konformitätsbewertung - Allgemeine Anforderungen an die Kompetenz von Referenzmaterialherstellern / General requirements for the competence of reference material producers
19. ISO Guide 34:2000; General requirements for the competence of reference material producers
20. DAkkS 71 SD 1/4 016; Ermittlung und Angabe der Messunsicherheit nach Forderungen der DIN EN ISO/IEC 17025 (2011) [Estimation and indication of the measurement uncertainty]
21. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Informationen für Verbraucher über das Nichtvorhandensein oder das reduzierte Vorhandensein von Gluten in Lebensmitteln / on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food
22. Taylor et al. (2014) Establishment of reference doses for residues of allergenic foods: report of the VITAL Expert Panel, Food Chem Toxicol 63: 9-17

23. Demmel et al. (2015) Kap. 4.1 Existierende Aktionswerte, in: Allergene in Lebensmitteln, Behr's Verlag, Hamburg [Chapter 4.1 Existing Action Levels, in Allergens in Foods]
24. VSEP (2019) Summary of the 2019 VITAL Scientific Expert Panel Recommendations, The Allergen Bureau Limited 2019, www.allergenbureau.net
25. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, CAC/GL 74-2010
26. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
27. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
28. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
29. Ministry of Health and Welfare, JSM, Japan 2006
30. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
31. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
32. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
33. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
34. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
35. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
36. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
37. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
38. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
39. ASU §64 LFGB L 16.01-9 Untersuchung von Lebensmitteln - Bestimmung von Soja (Glycine max) in Getreidemehl mittels real-time PCR (2016) [Foodstuffs, determination of soya (Glycine max) in cereal flour by real-time PCR]
40. ASU §64 LFGB L 18.00-19 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Sesam (Sesamum indicum) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of sesame (Sesamum indicum) in rice and wheat cookies and sauce powders by PCR]
41. ASU §64 LFGB L 18.00-20 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of almond (Prunus dulcis) in rice and wheat cookies and sauce powders by PCR]

42. ASU §64 LFGB L 18.00-21 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Paranuss (Bertholletia exceisa) in Reis- und Weizenkeksen sowe in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of brazil nut (Bertholletia exceisa) in rice and wheat cookies and sauce powders by PCR]
43. ASU §64 LFGB L 18.00-22 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]
44. ASU §64 LFGB L 08.00-59 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
45. ASU §64 LFGB L 08.00-64 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von von schwarzem Senf (Brassica nigra L.) und braunem Senf (Brassica juncea L.) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of black mustard (Brassica nigra L.) and brown mustard (Brassica juncea L.) in boiled sausages by real-time PCR]
46. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2017) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]
47. ASU §64 LFGB L 08.00-66 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Weizen (Triticum L.) und Roggen (Secale cereale) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of wheat (Triticum L.) and rye (Secale cereale) in boiled sausages by real-time PCR]