Evaluation Report
proficiency test

DLA 15/2019

Response PT Egg:

5 processed Samples Liquid Egg (pasteurized), Egg (boiled), Whole Egg Powder, Egg Pasta and Egg Pastry

in Potato Powder Matrix

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (8 February 2020) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 8 February 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Keine As part of the present proficency test the following services were subcontracted: none
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 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 whole egg powder 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 egg in liquid egg (pasteurized), egg (boiled), whole egg powder, egg pasta and egg pastry in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce processed egg products and raw eggs cooked by DLA (water, 10 min). For each PT-sample 4-8 products of different origin were worked up.

Premixes with contents from approx. 3,5 - 10 % of the regarding allergenic ingredients were produced (s. Tab. 1).

For this purpose, the products were, if necessary, freeze-dried (liquid egg, boiled eggs), crushed by means of a knife mill or centrifugal mill, mixed gravimetrically and homogenized. Except egg pastry and egg pasta the raw materials were then mixed with further ingredients and further crushed and homogenized using 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 egg of the PT-samples were in the range of 52 to 62 mg/kg as whole egg powder (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 egg 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** Egg Pastry	**Sample 2** Egg, cooked	**Sample 3** Whole Egg Powder	**Sample 4** Egg Pasta	**Sample 5** Liquid Egg	**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	74	75	75
Maltodextrin	25	25	25	24	25	25
Allergen-Premixes Ingredients: maltodextrin (<90%), silicon dioxide (<1,3%), processed allergen products (each 3,5% - 10% egg)	0,95	0,62	0,54	1,5	0,55	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Egg biscuits * (26% whole egg and sugar, wheat flour, invert sugar syrup, raising agent, aroma, glucose, salt)* Total protein 8,7 % *** *(6 Products, Europe)*	953	-	-	-	-	-
Eggs, cooked (freeze-dried)* Protein 49,4 % ** *(8 Products, Europe)*	-	61,5	-	-	-	-
*Whole egg powder** Protein 46,9 % ** *(6 Products, Europe)*	-	-	55,2	-	-	-
Egg pasta/noodles (15% whole egg and durum wheat, soft wheat, salt)* Total protein 13,6 % *** *(8 Products, Europe)*	-	-	-	1500	-	-
Liquid egg, pasteurized (freeze-dried)* Protein 50,0 % ** *(4 Products, Europe)*	-	-	-	-	55,1	-
– as Whole egg powder	59,9	61,5	55,2	51,7	55,1	-
Extended combined uncertainty (k=2) of egg content (= ± 11 %)	± 6,59	± 6,77	± 6,07	± 5,69	± 6,06	-

*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=6,25 for egg protein)
***Protein content calculated according to the declaration of the products (besides egg, other protein sources are included)
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 77%, 87%, 98%, 78% and 100%. 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 0,92, 0,77, 0,51, 0,91 and 0,46 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. 0,28 (21,6°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

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 16th week of 2019. The testing method was optional. The tests should be finished at May 31st 2019 the latest.

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

There are 5 different samples with similar contents of the allergenic parameter Egg, 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 Liquid Egg (pasteurized), Egg (boiled), Whole Egg Powder, Egg Pasta and Egg Pastry.*
- *Please give all your quantitative results as whole egg powder, if possible indicate the underlying total protein content in Egg.*
- *Possible conversion factors for processed Egg 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.

All 11 participants submitted the results in time.

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 egg in liquid egg (pasteurized), egg (boiled), whole egg powder, egg pasta and egg pastry, 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 Egg Pastry	Sample 2 Egg, cooked	Sample 3 Whole Egg Powder	Sample 4 Egg Pasta	Sample 5 Liquid Egg	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 for different parameters recovery rates in the range from 57% - 119% were obtained by ELISA methods and 33% - 145% 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[33-34].

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 [30]. 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 [30].

The IRMM (Institute for Reference Materials and Measurements) proved the suitability of five different ELISA-Kits for the determination of peanut [33]. The mean values were in the concentration range of 0,3 - 16,1 mg/kg and/or 1,2 - 20,4 mg/kg. The smallest relative reproducibility standard deviation for each Kit was obtained for dark chocolate at 20 - 42% and cookies at 23 - 61%.

Table 3b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations from experiments by precision and the resulting target standard deviation σ_{pt} [35-39].

Parameter	**Matrix**	**Mean [mg/kg]**	**Reco-very**	**rob RSD_r**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
Almond	Rice cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR multiplex ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR multiplex ASU 18.00-22
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soya flour	Boiled sausage (100°C, 60 min)	114,1 64,4	114 % 161 %	-	14,7% 27,7%	22,2% 41,4%	19,6% 36,5%	rt-PCR ASU 08.00-65
Soya flour	Sausage, autoclaved	33,1	33,1 %	-	21,5%	30,8	26,8%	rt-PCR ASU 08.00-65
Soya flour	Boiled sausage (100°C, 60 min)	82,0 39,6 19,6 9,3	82 % 99 % 98 % 93 %	-	17,3% 22,9% 22,9% 31,1%	24,1% 31,8% 24,0% 30,2%	20,8% 27,4% 17,7% -	rt-PCR ASU 08.00-59

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%.

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), PCR- and LC/MS 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.

ELISA-Results given as **egg white protein** or **egg protein (egg white and yolk proteins)** were converted to **whole egg powder**. When possible the information supplied by the test kit manufacturer was used. A content of 26 % egg white protein and 48 % egg protein in whole egg powder was taken.

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 Egg Products

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Probe 1 Egg Pastry	Probe 2 Egg, cooked	Probe 3 Whole Egg Powder	Probe 4 Egg Pasta	Probe 5 Liquid Egg	Probe 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5		
4	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
2	negative	positive	positive	positive	positive	negative	4 (80%)	BF	
11	negative	positive	positive	positive	positive	negative	4 (80%)	BK	
5a	positive	positive	positive	positive	positive	negative	5 (100%)	BK	
8	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
3	positive	positive	positive	positive	positive	negative	5 (100%)	MI-II	
7	positive	positive	positive	positive	positive	negative	5 (100%)	MI-II	
10a	positive	positive	positive	positive	positive	negative	5 (100%)	MI-II	
1	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
9	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
10b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
5b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	11	13	13	13	13	0
Number negative	2	0	0	0	0	13
Percent positive	85	100	100	100	100	0
Percent negative	15	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For the samples 2 to 5 consensus values of 100% positive results were obtained by the ELISA-methods. For the processed sample 1 (egg pastry) two negative results were obtained giving a consensus value of 85% positive results.

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

Evaluation number	Sample 1 Egg Pastry		Sample 2 Egg, cooked		Sample 3 Whole Egg Powder		Sample 4 Egg Pasta		Sample 5 Liquid Egg		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 **		
4	< 1,54		7,31	12	68,5	**124**	9,62	19	103	186	1/4 (25%)	AQ	results converted °
2	0,200	0,3	15,1	25	53,4	**97**	14,0	27	114	207	1/5 (20%)	BF	
11	< BG		21,0	34	56,1	**102**	9,00	17	74,0	**134**	2/4 (50%)	BK	
5a	2,70	4,5	23,2	38	56,0	**101**	13,9	27	74,1	**134**	2/5 (40%)	BK	
8	1,00	1,7	40,0	**65**	60,0	**109**	12,6	24	130	236	2/5 (40%)	IL	
3	39,2	**65**	24,2	39	42,7	**77**	46,0	**89**	50,8	**92**	4/5 (80%)	MI-II	results converted °
7	36,0	**60**	14,0	23	42,0	**76**	44,0	**85**	44,0	**80**	4/5 (80%)	MI-II	
10a	31,3	**52**	12,5	20	33,3	**60**	31,3	**60**	41,7	**76**	4/5 (80%)	MI-II	results converted °
1	2,60	4,3	6,60	11	56,5	**102**	11,1	21	83,8	152	1/5 (20%)	RS-F	
6	2,10	3,5	4,60	7	58,3	**106**	15,1	29	50,7	**92**	2/5 (40%)	RS-F	
9	2,29	3,8	12,6	20	71,1	**129**	18,2	35	99,9	181	1/5 (20%)	RS-F	
10b	2,00	3,3	15,0	24	58,0	**105**	19,0	37	80,0	**145**	2/5 (40%)	RS-F	
5b	3,04	5,1	7,80	13	66,6	**121**	16,7	32	92,2	167	1/5 (20%)	RS-F	
													° calculation p. 14
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	**3**	Number in RA	**1**	Number in RA	**13**	Number in RA	**3**	Number in RA	**7**			
	Percent in RA	**27**	Percent in RA	**8**	Percent in RA	**100**	Percent in RA	**23**	Percent in RA	**54**			

*Recovery rate 100% Reference value: Whole egg powder, see page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

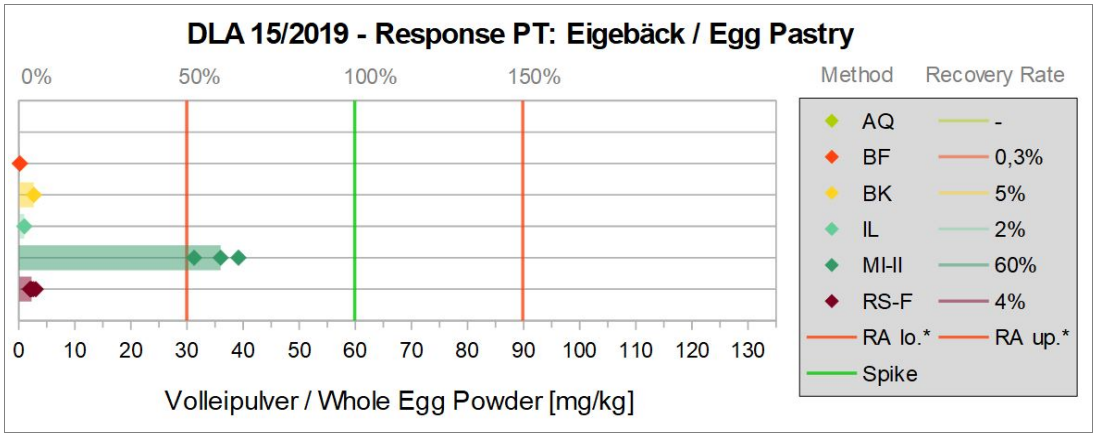
IL = Immunolab

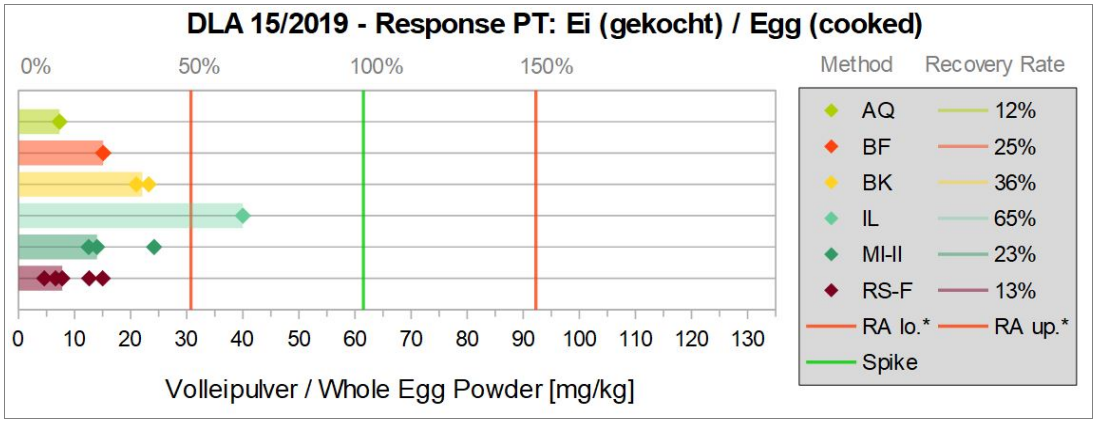
MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For sample 3 (whole egg powder) 100% of the recovery rates of the ELISA methods were in the range of acceptance of 50-150%. For liquid egg (pasteurized) (sample 5) 54% of the recovery rates of the participant results were in the range of acceptance and the other results were above. In contrast, a lower response was observed for sample 1 (egg pastries), sample 2 (boiled egg) and sample 4 (egg noodles) with 27% (3), 8% (1) and 23% (3). The other results were below the acceptance range.





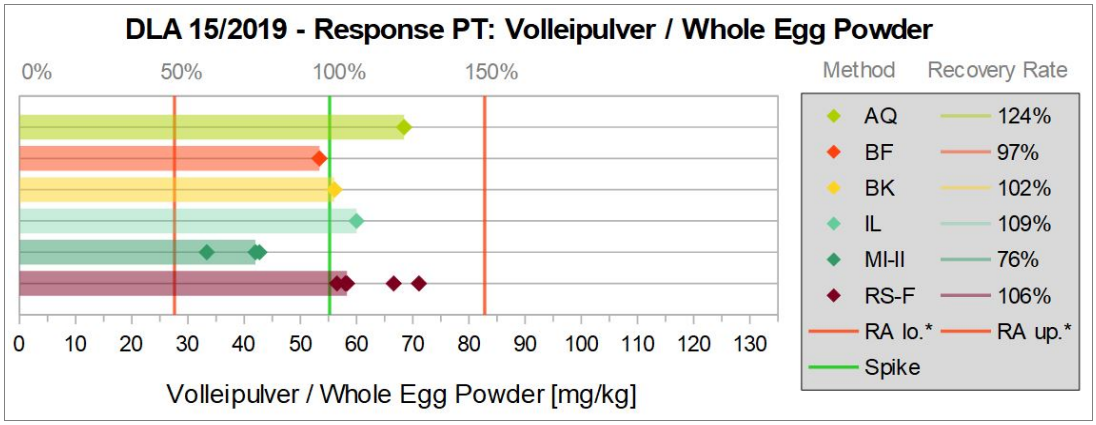


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale whole egg powder content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

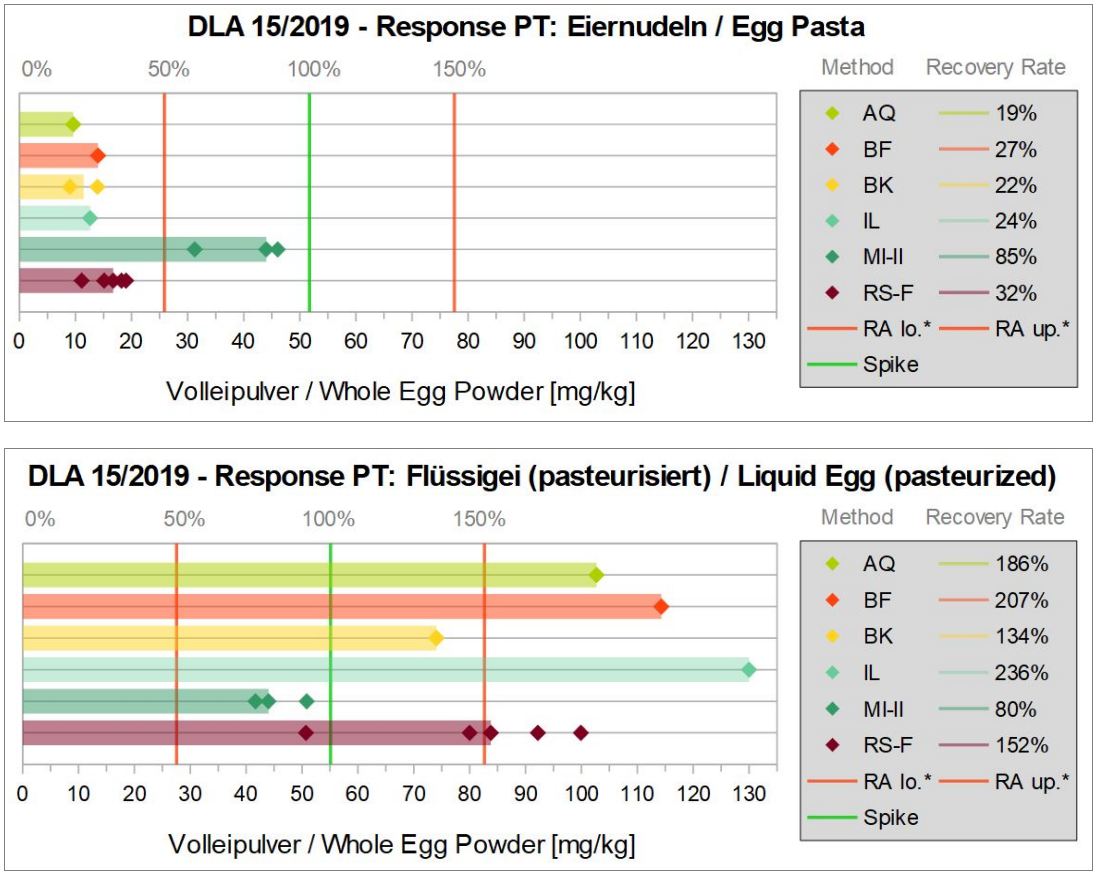


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale whole egg powder content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

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.	Evalu-ation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	preferred as almond
AQ	4	18.10.19	positive	<0,4 mg/kg	positive	1,9	positive	17,8	positive	2,5	positive	26,7	negative		0,05	0,4		egg white proteins, total
BF	2	08.11.19	negative	0,2	positive	15,1	positive	53,4	positive	14	positive	114,3	negative	0,3	0,3	1		whole egg powder
BK	11	08.10.19	-	< BG	-	21,0	-	56,1	-	9,0	-	74,0	-	< BG		1,5		whole egg powder
BK	5a	31.10.19	positive	2,7	positive	23,2	positive	56	positive	13,9	positive	74,1	negative		0,5	0,5		whole egg powder
IL	8	07.10.19	positive	1	positive	40	positive	60	positive	12,6	positive	130	negative	0	0,05	0,4		whole egg powder
MI-II	3	29.10/06.11	positive	18,8	positive	11,6	positive	20,5	positive	22,1	positive	24,4	negative	< 0,31	0,31	0,31		egg protein
MI-II	7	15.10.19	positive	36	positive	14	positive	42	positive	44	positive	44	negative	<0,65	0,65	0,65		whole egg protein
MI-II	10a	11.10.19	positive	15	positive	6	positive	16	positive	15	positive	20	negative		1,25	3,5	1,025	whole egg protein
RS-F	1	29./30.10.19	positive	2,6	positive	6,6	positive	56,5	positive	11,1	positive	83,8	negative		0,5	0,5		whole egg powder
RS-F	6	23.10.19	-	2,1	-	4,6	-	58,3	-	15,1	-	50,7	-	< 0,5	0,1	0,5		whole egg powder
RS-F	9	08.10.	positive	2,29	positive	12,6	positive	71,1	positive	18,2	positive	99,9	negative	< 0,5	0,1	0,5		whole egg powder
RS-F	10b	18.10.19	positive	2	positive	15	positive	58	positive	19	positive	80	negative		0,5	0,5	0,28	whole egg powder
RS-F	5b	31.10.19	positive	3,04	positive	7,8	positive	66,6	positive	16,7	positive	92,2	negative		0,5	0,5		whole egg powder

* 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 egg (According to method prescription)	Conversion for processed egg	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	4	AgraQuant ELISA Egg White COKAL0848, RomerLabs	polyclonal (egg white proteins)			as per kit instructions	yes	no conversion factors given by manufacturer
BF	2	MonoTrace Egg ELISA kit, BioFront Technologies	Monoclonal antibody-based assay	1:20 extraction ratio @ 62C for 10 minutes	N/A	Product # EOM-EK		
BK	11	BioKits Egg Assay Kit, Neogen	polyclonal Ab against Gal d1	10		as per kit instructions	yes	
BK	5a	BioKits Egg Assay Kit, Neogen	Ovomucoid		1 g Ovomucoid refers to 10 g egg white protein and 30 g whole egg powder	as per kit instructions	yes	
IL	8	Immunolab Egg white ELISA	Ovomucoid					
MI-II	3	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)		48,05 % total protein	Conversion factor from egg protein to egg powder is 2,06	Short Time Extraction Method	yes	
MI-II	7	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)	detects the egg white protein ovalbumin	hen's egg as whole egg powder contains 47,6% total protrein; factor 2,1	not given	as per kit instructions	yes	for our customers the results are being reported as mg/kg whole egg protein
MI-II	10a	Morinaga Egg (Ovalbumin) ELISA Kit II (M2111)					yes	
RS-F	1	Ridascreen® FAST Egg Protein R6402, R-Biopharm					yes	MU = 31,5%
RS-F	6	Ridascreen® FAST Egg Protein R6402, R-Biopharm		49 +/- 1		as per kit instructions	no	
RS-F	9	Ridascreen® FAST Egg Protein R6402, R-Biopharm				as per kit instructions	yes	
RS-F	10b	Ridascreen® FAST Egg Protein R6402, R-Biopharm					yes	
RS-F	5b	Ridascreen® FAST Egg Protein R6402, R-Biopharm	egg white proteins			as per kit instructions	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 15-2019 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	23,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,16	72	27,9
2	5,09	77	30,3
3	5,02	80	31,9
4	5,06	63	24,9
5	5,04	69	27,4
6	5,10	72	28,2
7	5,04	72	28,6
8	5,16	85	32,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	73,7	Particles
Standard deviation	6,57	Particles
χ^2 (CHI-Quadrat)	4,10	
Probability	**77**	%
Recovery rate	126	%

Normal distribution		
Number of samples	8	
Mean	29,0	mg/kg
Standard deviation	2,59	mg/kg
rel. Standard deviaton	8,91	%
Horwitz standard deviation	9,64	%
HorRat-value	**0,92**	
Recovery rate	126	%

Microtracer Homogeneity Test
DLA 15-2019 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	31,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	96	38,2
2	5,06	80	31,6
3	4,96	82	33,1
4	5,06	76	30,0
5	5,13	87	33,9
6	5,06	83	32,8
7	5,02	89	35,5
8	5,05	84	33,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	84,6	Particles
Standard deviation	6,18	Particles
χ^2 (CHI-Quadrat)	3,16	
Probability	**87**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	33,5	mg/kg
Standard deviation	2,45	mg/kg
rel. Standard deviaton	7,31	%
Horwitz standard deviation	9,43	%
HorRat-value	**0,77**	
Recovery rate	107	%

Microtracer Homogeneity Test
DLA 15-2019 Sample 3

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

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,18	92	35,5
2	5,02	99	39,4
3	5,01	95	37,9
4	5,16	92	35,7
5	5,12	90	35,2
6	5,05	99	39,2
7	5,07	93	36,7
8	5,14	100	38,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	95,0	Particles
Standard deviation	4,52	Particles
χ^2 (CHI-Quadrat)	1,50	
Probability	**98**	%
Recovery rate	120	%

Normal distribution		
Number of samples	8	
Mean	37,3	mg/kg
Standard deviation	1,77	mg/kg
rel. Standard deviaton	4,76	%
Horwitz standard deviation	9,28	%
HorRat-value	**0,51**	
Recovery rate	120	%

Microtracer Homogeneity Test
DLA 15-2019 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	26,5	mg/kg

Result of analysis

Sample	Einwaage [g]	Partikel Anzahl	Partikel [mg/kg]
1	5,18	74	28,6
2	5,16	73	28,3
3	5,05	68	26,9
4	5,10	80	31,4
5	5,13	86	33,5
6	5,11	68	26,6
7	5,06	75	29,6
8	5,02	82	32,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	75,8	Particles
Standard deviation	6,59	Particles
χ^2 (CHI-Quadrat)	4,01	
Probability	**78**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	29,7	mg/kg
Standard deviation	2,58	mg/kg
rel. Standard deviaton	8,70	%
Horwitz standard deviation	9,60	%
HorRat-value	**0,91**	
Recovery rate	112	%

Microtracer Homogeneity Test
DLA 15-2019 Sample 5

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

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	61	24,2
2	5,12	60	23,4
3	5,02	58	23,1
4	5,27	66	25,0
5	5,06	63	24,9
6	5,15	64	24,9
7	5,03	59	23,5
8	5,07	67	26,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	62,2	Particles
Standard deviation	2,81	Particles
χ^2 (CHI-Quadrat)	0,89	
Probability	**100**	%
Recovery rate	116	%

Normal distribution		
Number of samples	8	
Mean	24,4	mg/kg
Standard deviation	1,10	mg/kg
rel. Standard deviaton	4,52	%
Horwitz standard deviation	9,89	%
HorRat-value	**0,46**	
Recovery rate	116	%

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 15-2019***
PT name	**Response PT Egg: Processed samples Liquid Egg (pasteurized), Egg (boiled), Whole Egg Powder, Egg Pasta and Egg Pastry in potato powder matrix**
Sample matrix (processing)	***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: Egg / Egg Protein / DNA from Liquid Egg (pasteurized), Egg (boiled), Whole Egg Powder, Egg Pasta and Egg Pastry* *Samples 1-5: approx. 25 - 150 mg/kg (as whole egg powder)*
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 digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest November 08th 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		Germany
		USA
		Germany
		Germany
		Germany
		AUSTRIA
		Germany
		Germany
		Germany
		AUSTRIA

[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
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22. 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
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38. 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]
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