Proficiency Tests
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Evaluation Report
proficiency test

DLA 15/2018

Response PT Milk:

5 processed samples Milk, Whey, Yoghurt, Curd Cheese and Cream

in Potato Powder Matrix

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

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 16 October 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
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 total milk protein contents. The whey containing sample was adjusted to a higher milk protein content. 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 milk protein (casein, β-lactoglobulin) in milk, curd cheese, yoghurt, cream and whey in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce processed milk products. For each PT-sample 4-5 products of different origin were worked up. The water containing products were dried at 35-40°C prior to further use.
Afterwards premixes with contents from approx. 2,8 - 33 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed if necessary, mixed gravimetrically with further ingredients, crushed by a ball mill and homogenized.
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 milk protein of the PT-samples were in the range of 38 to 41 mg/kg and for the whey sample 63 mg/kg, respectively (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 milk 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** **Milk**	**Sample 2** **Curd Cheese**	**Sample 3** **Cream**	**Sample 4** **Yoghurt**	**Sample 5** **Whey**	**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	74	74	75	75
Maltodextrin	24	25	25	25	25	25
Allergen-Premixes Ingredients: maltodextrin (65% - 80%), titanium dioxide (< 25%), silicon dioxide (< 1,2-2%), processed allergen products (each 2,8% - 33% dry weight)	0,98	0,34	0,85	0,81	0,12	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Whole milk, dried** Protein 25,0 % ** *(5 products, Germany)*	154	-	-	-	-	-
*Curd cheese, dried** Protein 43,3 % ** *(5 products, Germany)*	-	95,4	-	-	-	-
*Cream, dried** Protein 6,33 % ** *(5 products, Germany)*	-	-	624	-	-	-
*Yoghurt, dried** Protein 25,0 % ** *(5 products, Germany)*	-	-	-	154	-	-
*Whey powder ** Protein 15,9 % ** *(4 products, Germany)*	-	-	-	-	398	-
- thereof Milk protein	38,6	41,3	39,5	38,6	63,3	-
Extended combined uncertainty (k=2) of milk protein content (= ± 11 %)	± 4,25	± 4,54	± 4,35	± 4,25	± 6,96	-

*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=6,38 for milk protein)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1 to 5 showed a probability of 98%, 80%, 68%, 95% and 98%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [18]. This gave a HorRat value of 0,59, 0,86, 0,85, 0,61 and 0,62 respectively. The results of 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. 0,23 (22°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 17th week of 2018. The testing method was optional. The tests should be finished at June 8th 2018 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 milk, 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 Milk, Whey, Yoghurt, Curd Cheese or Cream with known amounts of total milk protein, which is the base for the response comparison of the quantitative results of the participants.*
- *Please specify all your quantitative results as to what they refer to (e.g total milk protein).*
- *Possible conversion factors for processed milk 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 or protein 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.

11 participants submitted 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 milk (milk, yoghurt, cheese curd, cream and whey) were provided to determine the qualitative detectability and to determine the response in 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 Milk	Sample 2 Curd Cheese	Sample 3 Cream	Sample 4 Yoghurt	Sample 5 Whey	Sample 6 „blank"	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not sucessful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

3.2 Recovery-Score (RR-Score)

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

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

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

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

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 48% - 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 1 – 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} [35-38].

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9
Soya flour	Boiled saus-age (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 saus-age (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
Wheat + Rye	Boiled saus-age (100°C, 60 min)	96,1	120 %	-	21,3%	35,4%	32,0%	rt-PCR ASU 08.00-66
Wheat + Rye	Sausage, autoclaved	74,9	11,0 %	-	24,6%	32,7%	27,7%	rt-PCR ASU 08.00-66

3.2.2 Values by perception

Requirements to the performance of analysis methods for quantitative determination of allergens in food were compiled for example from the Ministry of Health and Welfare (MHLW) in Japan [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 [21]	**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-, 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 by recalculation to total milk protein and β-lactoglobulin content, respectively.

ELISA results given as casein were converted to milk protein (total) using the literature value of 80% casein in milk protein [39].
ELISA results given as skimmed milk powder were converted with a content of 35,1% milk protein (total) according to the regarding manufacturers kit specifications (Veratox, Neogen Allergen Handbook).

β-Lactoglobulin ELISA results given as milk protein (total) were converted using the literature value of 10% 10% β-lactoglobulin in milk protein [39].

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 Milk Products

4.1.1 Qualitative Scores: ElISA-Methods Milk Protein / Casein

Evaluation number	Sample 1 Milk	Sample 2 Curd Cheese	Sample 3 Cream	Sample 4 Yoghurt	Sample 5 Whey	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		
8	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
1a	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
2a	positive	positive	positive	positive	positive	negative	5 (100%)	AQ-P	
9a	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
7a	positive	positive	positive	positive	positive	negative	5 (100%)	MI	
3a	positive	positive	positive	positive	negative	negative	5 (100%)	RS-F C	
4a	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F C	
5a	positive	positive	positive	positive	negative	negative	5 (100%)	RS-F C	
5b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F C	
6b	positive	positive	positive	positive	negative	negative	5 (100%)	RS-F C	
11	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F C	
5c	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F M	
1c	positive	positive	positive	positive	positive	negative	5 (100%)	VT	
2b	positive	positive	positive	positive	positive	negative	5 (100%)	VT	
4b	positive	positive	positive	positive	positive	negative	5 (100%)	VT	
6c	positive	positive	positive	positive	positive	negative	5 (100%)	VT	
10	positive	positive	positive	positive	positive	negative	5 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	17	17	17	17	14	0
Number negative	0	0	0	0	3	17
Percent positive	100	100	100	100	82	0
Percent negative	0	0	0	0	18	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

AQ-P = AgraQuant Plus, RomerLabs

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F C= Ridascreen® Fast Casein, R-Biopharm

RS-F M= Ridascreen® Fast Milk, R-Biopharm

VT = Veratox, Neogen

Comments:

For all milk products (samples 1 to 5) consensus values of 100% positive results were obtained by the ELISA-methods specific for total milk protein or casein. For whey (sample 5) three negative results were obtained with the ELISA method RS-F casein. According to the test kit instructions, this method has no cross-reactivity to β-lactoglobulin, the main protein of the whey protein fraction.

4.1.2 Qualitative Scores: ELISA-Methods β-Lactoglobulin

Evaluation number	Sample 1 Milk	Sample 2 Curd Cheese	Sample 3 Cream	Sample 4 Yoghurt	Sample 5 Whey	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		
9b	-	-	-	-	positive	negative	1 (100%)	IL LG	
6a	positive	positive	positive	positive	positive	negative	5 (100%)	MI LG	
7b	positive	positive	positive	positive	positive	negative	5 (100%)	MI LG	
1b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F LG	
3b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F LG	

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

Methods:

IL LG= Immunolab β-Lactoglobulin

MI LG= Morinaga Institute β-Lactoglobulin ELISA

RS-F LG= Ridascreen® Fast β-Lactoglobulin, R-Biopharm

Comments:

For all milk products (samples 1 to 5) consensus values of 100% positive results were obtained by the ELISA-methods specific for β-lactoglobulin.

4.1.3 Qualitative Scores: LC/MS-Methods

Evaluation number	Sample 1 Milk	Sample 2 Curd Cheese	Sample 3 Cream	Sample 4 Yoghurt	Sample 5 Whey	Sample 6 „Blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
2	positive	positive	positive	positive	positive	negative	5/5 (100%)	LC/MS	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Spiking	positive	positive	positive	positive	positive	negative

Methods:

LC/MS = Liquid chromatography / Mass spectrometry

Comments:

For all milk products (samples 1 to 5) positive results were obtained by the LC/MS-method.

4.1.4 Quantitative Recovery-Rates Scores (RR-Scores): ELISA-Methods Milk Protein / Casein

Given as Milk Protein (total)

Evaluation number	Sample 1 Milk		Sample 2 Curd Cheese		Sample 3 Cream		Sample 4 Yoghurt		Sample 5 Whey		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**		
8	59,9	155	39,6	**96**	30,8	**78**	31,7	**82**	7,30	12	3/5 (60%)	AQ	
1a	> 30		13,3	32	26,8	**68**	24,8	**64**	0,81	1,3	2/5 (40%)	AQ	result converted °
2a	6,75	17	5,00	12	12,0	30	6,50	17	3,63	5,7	0/5 (0%)	AQ-P	result converted °
9a	59,0	153	54,0	**131**	52,0	**132**	60,0	155	32,0	**51**	3/5 (60%)	IL	
7a	25,0	**65**	36,3	**88**	21,3	**54**	27,5	**71**	1,88	3,0	4/5 (80%)	MI	result converted °
3a	30,0	**78**	45,0	**109**	25,0	**63**	35,0	**91**	<1,7		4/5 (80%)	RS-F C	result converted °
4a	37,0	**96**	63,0	153	34,0	**86**	25,0	**65**	1,90	3,0	3/5 (60%)	RS-F C	
5a	27,6	**72**	30,6	**74**	24,3	**61**	24,8	**64**	< 3,1		4/5 (80%)	RS-F C	result converted °
5b	23,3	**60**	34,9	**84**	22,3	**56**	21,5	**56**	1,35	2,1	4/5 (80%)	RS-F C	result converted °
6b	63,5	165	42,1	**102**	45,0	**114**	66,4	172			2/5 (40%)	RS-F C	result converted °
11	45,4	**118**	67,1	162	32,0	**81**	32,1	**83**	1,40	2,2	3/5 (60%)	RS-F C	
5c	35,8	**93**	47,1	**114**	35,9	**91**	28,8	**75**	212	335	4/5 (80%)	RS-F M	
2b	7,30	19	2,10	5,1	> 8,8		5,51	14	> 8,8		0/5 (0%)	VT	result converted °
6c	5,34	14	2,81	6,8	10,7	27	5,09	13	20,7	33	0/5 (0%)	VT	result converted °
10	23,2	**60**	11,8	28,6	29,6	**75**	22,5	**58**	102	161	3/5 (60%)	VT	

° Calculation p. 14

RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %
number in RA	8	number in RA	8	number in RA	12	number in RA	10	number in RA	1
percent in RA	57	percent in RA	53	percent in RA	86	percent in RA	67	percent in RA	9

*Recovery rate 100% Reference value: Milk protein, see page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs
AQ-P = AgraQuant Plus, RomerLabs
IL = Immunolab
MI = Morinaga Institute ELISA
RS-F C= Ridascreen® Fast Casein, R-Biopharm
RS-F M= Ridascreen® Fast Milk, R-Biopharm
VT = Veratox, Neogen

Comments:

For the milk product samples 1 to 4 53-86% of the recovery rates of participants' results obtained by milk protein and casein specific ELISA methods were in the range of acceptance of 50-150%. For the whey product (sample 5) one recovery rate was in the range of acceptance.

4.1.5 Quantitative Recovery Rates-Scores (RR-Scores): ELISA-Methods β-Lactoglobulin

Given as β-Lactoglobulin

Evaluation number	Sample 1 Milk		Sample 2 Curd Cheese		Sample 3 Cream		Sample 4 Yoghurt		Sample 5 Whey		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**		
9b	-	-	-	-	-	-	-	-	31,0	**98**	1/1 (100%)	IL LG	
6a	1,57	41	2,94	**71**	1,51	38	1,50	39	13,6	43	1/5 (20%)	MI LG	result converted °
7b	2,30	**60**	2,60	**63**	2,00	**51**	1,60	41	15,0	47	3/5 (60%)	MI LG	
1b	2,27	**59**	2,55	**62**	2,26	**57**	2,23	**58**	> 4,5		4/4 (100%)	RS-F LG	
3b	1,90	49	3,50	**85**	2,10	**53**	2,00	**52**	12,0	38	3/5 (60%)	RS-F LG	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	number in RA	**3**	number in RA	**4**	number in RA	**3**	number in RA	**2**	number in RA	**1**			
	percent in RA	**75**	percent in RA	**100**	percent in RA	**75**	percent in RA	**50**	percent in RA	**25**			

° Calculation p. 14

Methods:
IL LG= Immunolab β-Lactoglobulin
MI LG= Morinaga Institute β-Lactoglobulin ELISA
RS-F LG= Ridascreen® Fast β-Lactoglobulin, R-Biopharm

*Recovery rate 100% Reference value: β-Lactoglobulin = 10% (samples 1-4) and 50% (sample 5) from total milk protein, see page 6

** Acceptance range of AOAC for allergen ELISAs

Comments:

For the milk product samples 1 to 4 50-100% of the recovery rates of participants' results obtained by β-lactoglobulin specific ELISA methods were in the range of acceptance of 50-150%. For the whey product (sample 5) one recovery rate was in the range of acceptance.

4.1.6 Quantitative Recovery Rates-Scores (RR-Scores): LC/MS-Methods

Given as Milk Protein (total)

Evaluation number	Sample 1 Milk		Sample 2 Curd Cheese		Sample 3 Cream		Sample 4 Yoghurt		Sample 5 Whey		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	number in RA**		
2	35,6	92	54,6	132	30,0	76	49,6	128	< LOQ		4/5 (80%)	LC/MS	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	number in RA	1	number in RA	1	number in RA	1	number in RA	1	number in RA	0			
	percent in RA	100	percent in RA	100	percent in RA	100	percent in RA	100	percent in RA	0			

*Recovery rate 100% Reference value: Milk protein, see page 6

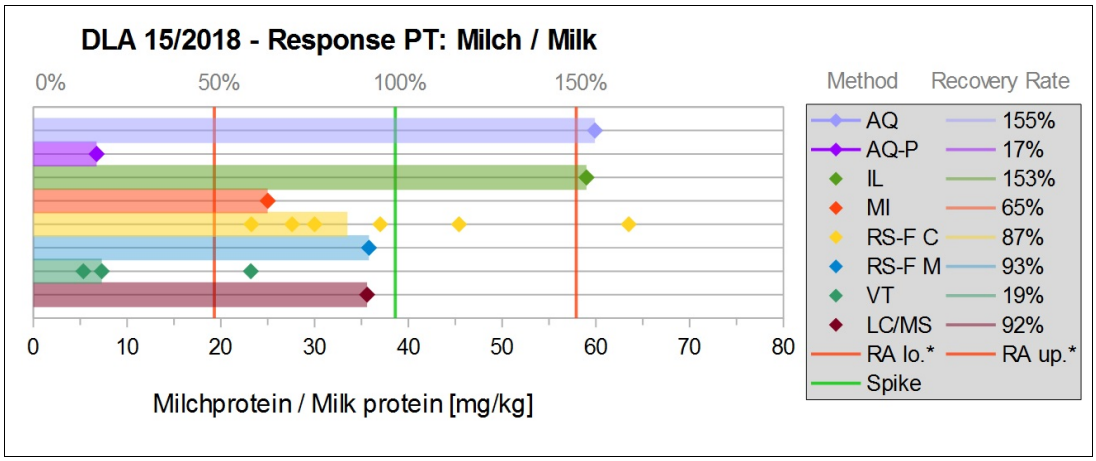
** Acceptance range of AOAC for allergen ELISAs

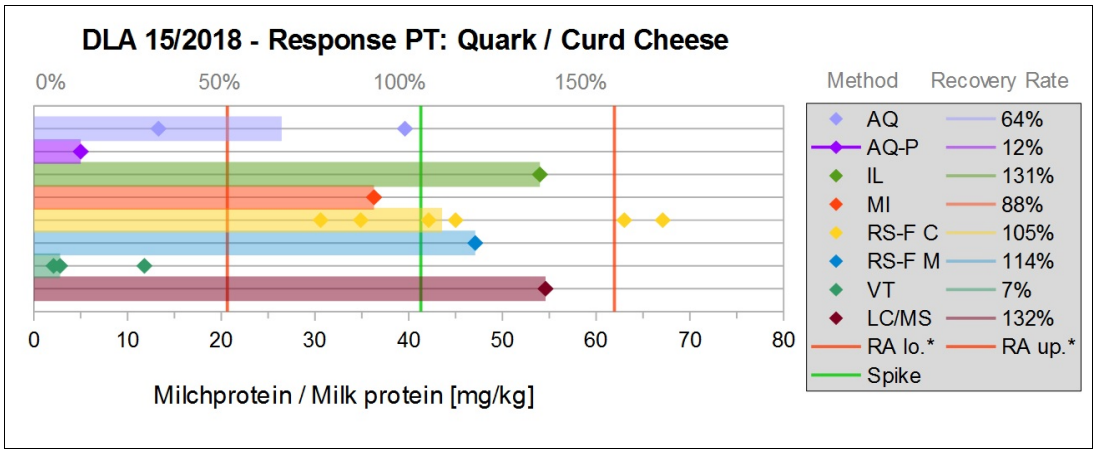
Methods:

LC/MS = Liquid chromatography / Mass spectrometry

Comments:

For the milk product samples 1 to 4 all recovery rates of participants' results were in the range of acceptance of 50-150%. For the whey product (sample 5) the result was below the limit of quantitation. According to the details given by the participant the LC/MS method is specific for casein (see documentation), which is not a whey protein.





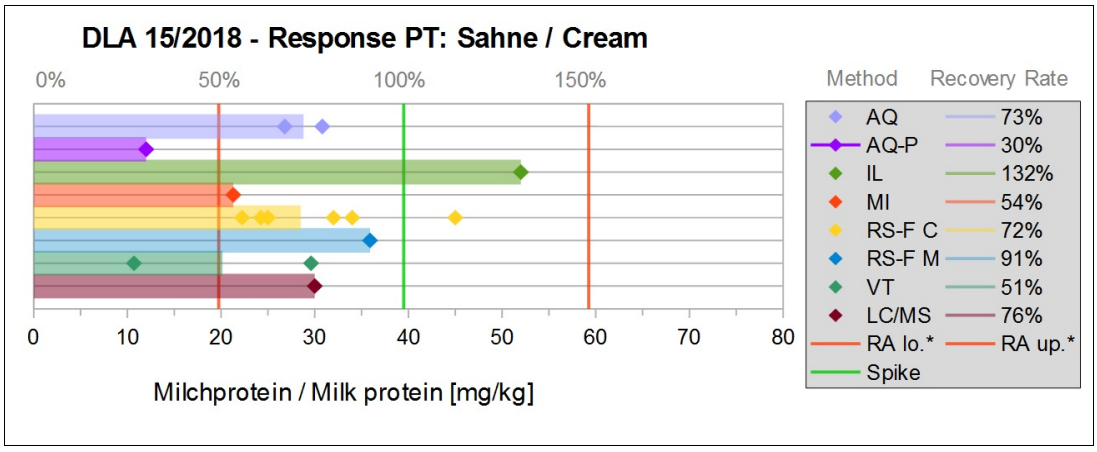
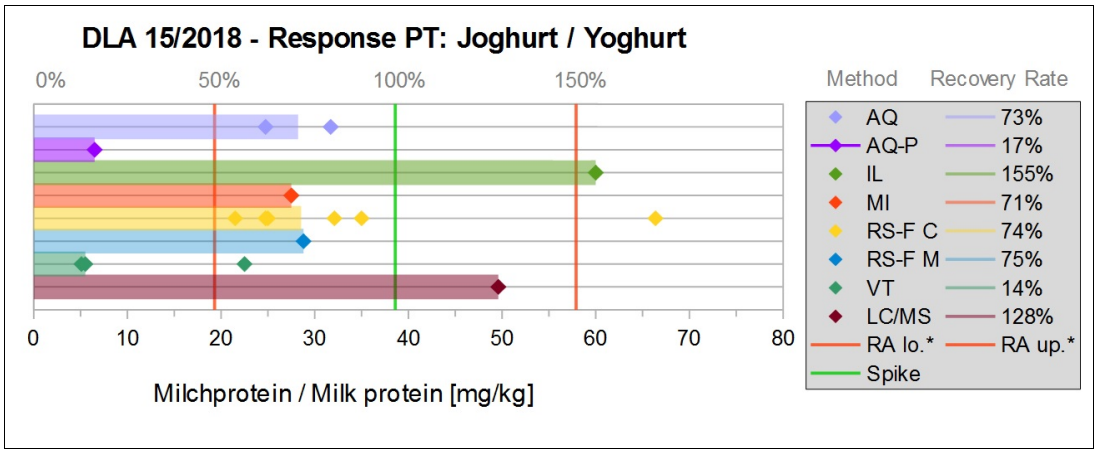


Abb./Fig. 1: Graphs of single results as milk protein (total) (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale milk protein content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper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



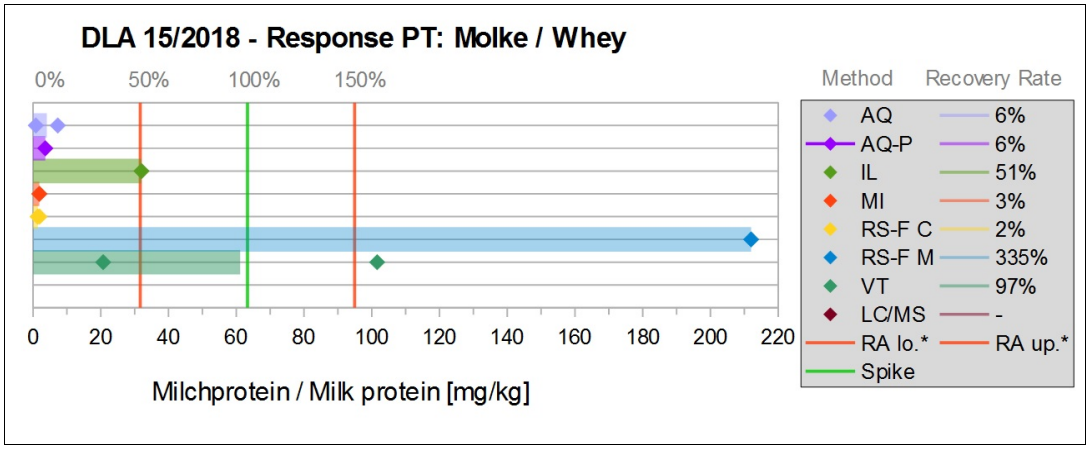


Abb./Fig. 2: Graphs of single results as milk protein (total) (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale milk protein content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper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

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		LOD	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 Milk Protein
AQ	8	07.05.18	positive	59,9	positive	39,6	positive	30,8	positive	31,7	positive	7,3	negative	<LOD	0,05	0,4	0,25	Milk proteins, total
AQ	1a	17.05.18	positive	> 24	positive	10,6	positive	21,4	positive	19,8	positive	0,65	negative	< 0.2	0,2			Casein
AQ-P	2a	22.05.18	positive	5,4	positive	4	positive	9,6	positive	5,2	positive	2,9	negative	< 1		1		Casein
IL	9a	05.02.18	positive	59	positive	54	positive	52	positive	60	positive	32	negative	0	0,05	0,4		Milk proteins, total
MI C	7a		positive	20	positive	29	positive	17	positive	22	positive	1,5	negative	<0,25	0.25	0,25		Casein
RS-F C	3a	07.06.18	positive	24	positive	36	positive	20	positive	28	negative	<1,36	negative	<1,36	1,36	2,5		Casein
RS-F C	4a	07.06.	positive	37	positive	63	positive	34	positive	25	positive	1,9	negative		0,6	0,6		Milk proteins, total
RS-F C	5a	06.06.	positive	22,1	positive	24,5	positive	19,4	positive	19,8	negative	< 2,5	negative	< 2,5	0,71	2,5		Casein
RS-F C	5b	06.06.	positive	18,6	positive	27,9	positive	17,8	positive	17,2	positive	1,08	negative	< 0,5	0,12	0,5		Casein
RS-F C	6b	18.05.18	positive	50,8	positive	33,7	positive	36	positive	53,1	negative		negative		2,5	2,5	0,5	Casein
RS-F C	11	27.06.18	positive	45,4	positive	67,1	positive	32	positive	32,1	positive	1,4	negative	<0.5	0,12	0,5		Milk proteins, total
RS-F C	11	27.06.18	positive	36,3	positive	53,7	positive	25,6	positive	25,7	positive	1,1	negative	<0.5	0,12	0,5		Casein
RS-F M	5c	05.06.	positive	35,8	positive	47,1	positive	35,9	positive	28,8	positive	212	negative	< 2,5	0,7	2,5		Milk proteins, total
VT	1c	07.05.18	positive		positive		positive		positive		positive		negative	< 2.5	2,5			Skimmed milk powder
VT	2b	03.05.18	positive	20,8	positive	6,0	positive	>25	positive	15,7	positive	> 25	negative	< 2,5		2,5	0,4	Skimmed milk powder
VT	4b	06.06.	positive		positive		positive		positive		positive		negative		0,9			Milk proteins, total

Continuation details by participants: ELISA-Methods Milk Protein / Casein

Method Abr.	Evaluation Number	Method	Specificity	Conversion for processed milk products / proteins	Remarks to the Method (Extraction and Determination)	Method accr. 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	8	AgraQuant ELISA Milk COKAL2448, RomerLabs	milk proteins, total		aquaous buffer/15 min/60°C	yes	
AQ	1a	AgraQuant Casein COKAL 1200, RomerLabs				yes	
AQ-P	2a	AgraQuant Plus Casein COKAL1248F, RomerLabs			As per kit instructions	no	for sample 2: 4,0, is automatically rounded, for dilution slightly higher contents (single determination)
IL	9a	Immunolab Milk ELISA					
MI C	7a	Morinaga Casein ELISA Kit II M2113	Cow's Milk Casein	Casein * 1,25 = total milk protein	As per kit instructions	yes	mg/kg total milk protein sample 1: 25, sample 2: 36, sample 3: 21, sample 4: 28, sample 5: 1,875, sample 6: <0,31
RS-F C	3a	R4612 RIDASCREEN FAST Casein R-Biopharm					
RS-F C	4a			Milk proteins total=Casein/0,8	Extraction 9.1	yes	Ridascreen Fast Casein (R4612)
RS-F C	5a	Ridascreen® FAST Casein R4612, R-Biopharm			Extraction according to kit instructions with Extractor 2	yes	
RS-F C	5b	Ridascreen® FAST Casein R4612, R-Biopharm			Extraction according to kit instructions with extraction buffer	yes	
RS-F C	6b			Cow's milk contains approx. 2,56 % Casein	according to handbook chapter 9.2 (1 g sample weight + 4 ml extractor 2 + 16 ml A-AEP)	yes	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F C	11	Ridascreen fast Casein		Assumption: Casein represents 80% of total Milk Protein		yes	
RS-F C	11	Ridascreen fast Casein				yes	
RS-F M	5c	Ridascreen® FAST Milk R4652, R-Biopharm			Extraction according to kit instructions	yes	
VT	1c	Veratox Total Milk Allergen, Neogen				yes	
VT	2b	Veratox Total Milk Allergen, Neogen			As per kit instructions	yes	for sample 2: 6,0, is automatically rounded
VT	4b	Veratox Total Milk Allergen, Neogen				yes	
VT	6c	Veratox Total Milk Allergen, Neogen	Antibodies against Casein & Whey protein		5 g sample weight according to handbook	yes	
VT	10	Veratox Total Milk Allergen, Neogen				yes / no	

5.1.2 ELISA-Methods (β-Lactoglobulin)

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		LOD	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 Milk Protein
IL LG	9b		-		-		-		-		positive	31	-					beta-Lactoglobulin
MI LG	6a	24.05.18	positive	15,7	positive	29,4	positive	15,1	positive	15	positive	135,9	negative		0,6	1,25	0,52	milk proteins, total
MI LG	7b		positive	2,3	positive	2,6	positive	2	positive	1,6	positive	15	negative	<0,031	0,031	0,031		beta-Lactoglobulin
RS-F LG	1b	09.05.18	positive	2,27	positive	2,55	positive	2,26	positive	2,23	positive	> 4.5	negative	< 0.167	0,167			beta-Lactoglobulin
RS-F LG	3b	07.06.18	positive	1,9	positive	3,5	positive	2,1	positive	2,0	positive	12	negative	<0,04	0,04	0,167		beta-Lactoglobulin

Method Abr.	Evaluation Number	Method	Specificity	Conversion for processed milk products / proteins	Remarks to the Method (Extraction and Determination)	Method accr.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	
IL LG	9b	Immunolab ß-Lactoglobulin ELISA					
MI LG	6a	andere: bitte eingeben!	anti-beta-lactoglobulin polyclonal antibody		1 g sample weight according to handbook	yes	MIoBS Beta-lactoglobulin ELISA KitII (M2112)
MI LG	7b	Morinaga ßLac ELISA Kit II M2112	Cow's milk ßLac	whey protein * 10 = total milk protein	As per kit instructions	yes	mg/kg total milk protein sample 1: 23, sample 2: 26, sample 3: 20, sample 4: 16, sample 5: 150 (probably spiked with whey), sample 6: <0,31
RS-F LG	1b	Ridascreen® FAST β-Lactoglobulin R4912, R-Biopharm				yes	
RS-F LG	3b	RIDASCREEN® FAST β-Lactoglobulin (Art. No. R4902)					

5.1.3 LC/MS-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		LOD	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 Milk Protein
LC/MS	2		positive	35,6	positive	54,6	positive	30,0	positive	49,6	positive	< LOQ	negative	< LOQ	2	4		Milk protein

Method Abr.	Evaluation Number	Method	Specificity	Conversion for processed milk products / proteins	Remarks to the Method (Extraction and Determination)	Method accr.to ISO / IEC 17025	Further remarks
		Test-Kit + Provider		Recalculation from X to Y (factor or %)	e.g. Extraction solution / time / temperature	yes/no	
LC/MS	2	LC-MS/MS	alpha S1-casein	alpha S1-casein in total protein: 32,4%	aqueous protein extraction, followed by tryptic digestion, clean-up of peptide extracts, detection of milk specific peptide sequences (alpha S1-casein) by UHPLC-MS/MS, extrapolation to milk protein, quantification by isotope-labeled peptide standards and solvent calibration	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 15-2018 Sample 1

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	72	28,7
2	5,05	67	26,5
3	4,97	72	29,0
4	5,01	63	25,1
5	5,00	64	25,6
6	5,05	64	25,3
7	5,03	69	27,4
8	5,01	64	25,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	66,9	Particles
Standard deviation	3,84	Particles
χ^2 (CHI-Quadrat)	1,55	
Probability	**98**	%
Recovery rate	117	%

Normal distribution		
Number of samples	8	
Mean	26,7	mg/kg
Standard deviation	1,53	mg/kg
rel. Standard deviaton	5,75	%
Horwitz standard deviation	9,76	%
HorRat-value	**0,59**	
Recovery rate	117	%

Microtracer Homogeneity Test
DLA 15-2018 Sample 2

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	84	33,6
2	5,05	73	28,9
3	5,01	86	34,3
4	5,02	82	32,7
5	5,02	86	34,3
6	5,03	92	36,6
7	5,06	76	30,0
8	5,00	75	30,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	81,8	Particles
Standard deviation	6,68	Particles
χ^2 (CHI-Quadrat)	3,82	
Probability	**80**	%
Recovery rate	115	%

Normal distribution		
Number of samples	8	
Mean	32,5	mg/kg
Standard deviation	2,66	mg/kg
rel. Standard deviaton	8,17	%
Horwitz standard deviation	9,47	%
HorRat-value	**0,86**	
Recovery rate	115	%

Microtracer Homogeneity Test
DLA 15-2018 Sample 3

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	44,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	122	49,0
2	4,98	133	53,4
3	4,97	118	47,5
4	5,04	117	46,4
5	5,01	108	43,1
6	4,97	114	45,9
7	5,00	120	48,0
8	4,98	104	41,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	117,0	Particles
Standard deviation	8,96	Particles
χ^2 (CHI-Quadrat)	4,81	
Probability	**68**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	46,9	mg/kg
Standard deviation	3,59	mg/kg
rel. Standard deviaton	7,66	%
Horwitz standard deviation	8,97	%
HorRat-value	**0,85**	
Recovery rate	104	%

Microtracer Homogeneity Test
DLA 15-2018 Sample 4

Weight whole sample	1,01	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	Einwaage [g]	Partikel Anzahl	Partikel [mg/kg]
1	5,03	90	35,8
2	5,00	95	38,0
3	5,05	101	40,0
4	5,00	96	38,4
5	5,06	105	41,5
6	5,04	95	37,7
7	5,02	91	36,3
8	5,03	104	41,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	97,1	Particles
Standard deviation	5,42	Particles
χ^2 (CHI-Quadrat)	2,12	
Probability	**95**	%
Recovery rate	116	%

Normal distribution		
Number of samples	8	
Mean	38,6	mg/kg
Standard deviation	2,16	mg/kg
rel. Standard deviaton	5,59	%
Horwitz standard deviation	9,23	%
HorRat-value	**0,61**	
Recovery rate	116	%

Microtracer Homogeneity Test
DLA 15-2018 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	19,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	62	24,6
2	5,01	55	22,0
3	5,03	52	20,7
4	4,99	54	21,6
5	4,98	51	20,5
6	5,02	52	20,7
7	5,06	53	20,9
8	4,98	53	21,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,0	Particles
Standard deviation	3,35	Particles
χ^2 (CHI-Quadrat)	1,46	
Probability	**98**	%
Recovery rate	109	%

Normal distribution		
Number of samples	8	
Mean	21,5	mg/kg
Standard deviation	1,34	mg/kg
rel. Standard deviaton	6,21	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,62**	
Recovery rate	109	%

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 15-2018***
PT name	***Response PT Milk: Processed samples Milk, Whey, Yoghurt, Curd Cheese and Cream in potato powder matrix*** *(levels: 25 - 150 mg/kg)*
Sample matrix (processing)	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (air dried, 40°C) (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:* ***Milk / Milkprotein(s) / bovine DNA*** ***Milk, Whey, Yoghurt, Curd Cheese*** *and* ***Cream*** *Samples 1-5: approx. 25 - 150 mg/kg (as total milk protein)*
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 June 08th 2018***
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. 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
		SWITZERLAND
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[The address data of the participants were deleted for publication of the evaluation report.]

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