Proficiency Tests
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Evaluation Report
proficiency test

DLA 09/2018

Allergens IX:

Milk (Casein) and Egg White Proteins

in Wine

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

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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 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].

2. Realisation

2.1 Test material

Two PT-samples with the same food matrix were provided for the detection and quantitative determination of the allergens in the range of mg/kg as well as one spiking level sample with a simple matrix. One of the samples (spiked sample) and the spiking level sample contain the respective allergenic ingredients in a similar concentration range. The results of the spiking level sample should give the possibility of a comparison with the spiked sample in respect to the detectability of the allergens with and without the influence of matrix and / or food processing.

The food matrix test material is a common in commerce white wine "Grauer Burgunder" (Baden, German quality wine). The basic composition of both sample A and sample B was the same (see table 1). The pH value of the wine was adjusted to pH 7-8 in order to stabilize the allergens in solution.

Afterwards the **spiked sample A** was produced as follows:
The spiking materials (premix) containing the allergenic ingredients skimmed milk powder and egg white powder (wine treatment agent) were solved in the basic mixture and the mixture was homogenized.

For the **spiking level sample,** the allergenic compounds above mentioned were added during a multi-stage addition of glucose and homogenization. Afterwards the total sample was sieved (mesh 400 µm) and homogenized again.

The samples A and B were portioned to approximately 50 ml in PE-bottles with screw lock, the spiking level sample to approximately 15 g in metallized PET film bags.

Table 1: Composition of the DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
White Wine Grauer Burgunder Labelling: Grauer Burgunder dry, German quality wine, Baden, contains sulfites,12,5 % vol Pre-treatment: pH adjusted with sodium carbonate solution to pH 7-8	99,7 g/100 g	100 g/100g	-
Glucose	-	-	99,8 g/100 g
Milk: - as Skimmed Milk Powder* - *thereof 37,6% total protein*** - *thereof Casein**** - *thereof β-Lactoglobulin****	 233 mg/kg 87,6 mg/kg 70,1 mg/kg 8,8 mg/kg	-	 276 mg/kg 104 mg/kg 83,2 mg/kg 10,4 mg/kg
Egg White Powder (Wine Treatment Agent): Ingredients: Hen's egg white (pasteurized, spray dried) - as Egg White Powder* - *thereof 76,4% total protein** *(egg white protein)*	 70,0 mg/kg 53,5 mg/kg	-	 83,0 mg/kg 63,4 mg/kg
further Ingredients: Maltodextrin, sodium chloride, sodium sulfate and silicon dioxide	<0,35 g/100 g	-	<0,35 g/100 g

*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=6,38 for milk protein and F=6,25 for egg protein)
*** Protein calculated according to literature (approx. 80% caseine and approx. 10% β-lactoglobulin in total milk protein [29]

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].
Because only powdered samples can be analysed be the applied microtracer method, only the spiking level sample was measured. The microtracer analysis of the present PT sample showed a probability of 99%. 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 a HorRat value of 0,57. The results of microtracer analysis are given in the documentation.

The **homogeneity of the bottled DLA samples** (spiked sample A) was tested by ELISA for the contents of casein and ovalbumin (see next page). The resulting standard deviations between the samples of < 15% were considered sufficient for the applied methods [18, 19, 22, 23].

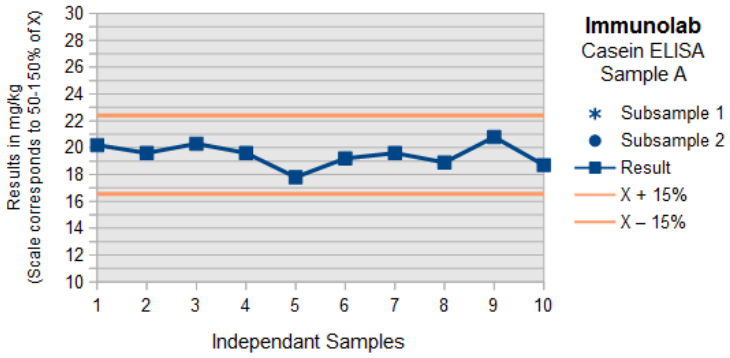
In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.6 and 3.8) [3].

ELISA-Tests: Homogenität Milch / Homogeneity Milk

Immunolab Casein ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Casein 19,5 ± 0,9 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg
1	-	-	20,2
2	-	-	19,6
3	-	-	20,3
4	-	-	19,6
5	-	-	17,8
6	-	-	19,2
7	-	-	19,6
8	-	-	18,9
9	-	-	20,8
10	-	-	18,7
	General average X		19,5
	SD of samples		0,87
			4,5%

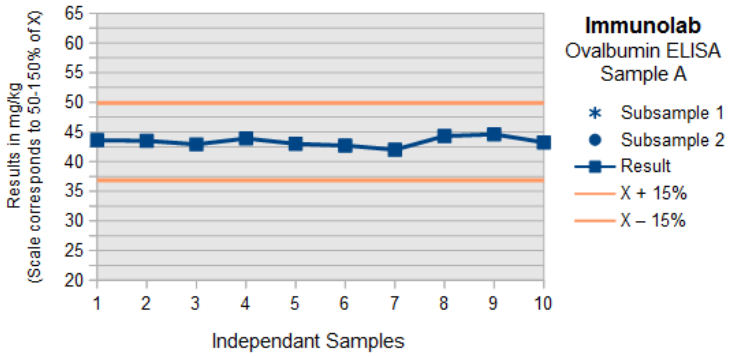


ELISA-Tests: Homogenität Ei / Homogeneity Egg

Immunolab Ovalbumin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Ovalbumin 43,4 ± 0,8 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg
1	-	-	43,6
2	-	-	43,5
3	-	-	42,9
4	-	-	43,9
5	-	-	43,0
6	-	-	42,7
7	-	-	42,0
8	-	-	44,3
9	-	-	44,6
10	-	-	43,2
	General average X		43,4
	SD of samples		0,78
			1,8%



2.1.2 Stability

The food matrix sample material is wine. In own long-term stability tests over two years, the parameter egg white proteins has proved to be stable, while casein levels have decreased (ELISA determinations). Over the short-term period of the PT no decrease was observed. However, on the basis of these findings, the evaluation of the participants results for the parameter casein in wine can be carried out with additional consideration of the standard uncertainty of the assigned value by means of z'-score.

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 spiking level sample was approx. 0,30 (25,3°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 materials sample A, B and the spiking level sample were sent to every participating laboratory in the 5th week of 2018. The testing method was optional. The tests should be finished at February 16th March 2018.

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

*There are two different samples A and B possibly containing the allergenic parameters **milk (casein)** and/or **egg white protein** in the range of mg/kg in the matrix **white wine**. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The **"spiking level sample"** contains the allergens in a simple matrix in **similar amounts** without further processing.*

***Important Note:** The pH-value of the wine samples A and B was adjusted with a sodium carbonate solution to pH 7-8, in order to stabilize the allergens in solution/suspension. Before analysis we recommend to shake the wine samples gently.*

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.
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.

All participants submitted their results in time. One registration was cancelled before sample shipment.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [25, 26, 27, 28]. It is for this reason that we contrast the results of the present proficiency test with several assigned values.
Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.

For quantitative results of the spiking material sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

ELISA- and PCR results were valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

If possible, this is the standard procedure for the evaluation of ELISA methods for the determination of allergens:

i) ***Assigned value of all results*** - $X_{pt_{ALL}}$
ii) ***Assigned value of single methods*** - $X_{pt_{METHOD\ i}}$
with at least 5 quantitative results given.

Single results giving values outside the measuring range of the participating laboratory or given as „0" are not considered for statistical evaluation (e.g. results given as > 25 mg/kg and < 2,5 mg/kg, respectively) [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^x) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

The following robust standard deviations were considered:

i) ***Robust standard deviation of all results*** - S^x_{ALL}
ii) ***Robust standard deviation of single methods*** - $S^x_{METHOD\ i}$
with at least 5 quantitative results given.

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.4 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.
In the present PT the target standard deviation was determined according to 3.4.3 value by perception.

3.4.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

The target standard deviation according to Horwitz is currently not achievable by ELISA or PCR-methods for values in the mg/kg range and was therefore not considered for evaluation.

3.4.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative trial or proficiency test) the target standard deviation σ_{pt} can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2\left(m - 1/m\right)}$$

The precision data in table 2 were obtained in collaborative trials with spiked wine samples by ELISA testkit methods, some of them modified [31, 32, 34]. Depending on the allergen amount relative reproducibility standard deviations were 12 – 36 % in the range of > 1 mg/L and 14 - 90 % in the range of < 1 mg/L.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments [31, 32, 34]

Parameter	**Matrix**	**Mean**	**RSD_r**	**RSD_R**	**Method / Literature**
Caseinate	White wines	0,057 – 0,78 mg/L	-	35,1 – 90,0 %	ELISA [31]
Caseinate	White wines	1,4 – 3,0 mg/L	-	20,3 – 29,4 %	ELISA [31]
Caseinate	White wines	6,3 – 6,8 mg/L	-	12,1 – 21,4 %	ELISA [31]
Egg white proteins	Red wines	1,0 – 1,4 mg/L	23,0 - 27,6 %	30,6 – 32,9 %	ELISA [32]
Egg white proteins	Red wines	3,5 – 4,2 mg/L	14,7 – 19,3 %	26,2 – 31,1 %	ELISA [32]
Egg white proteins	Red wines	5,9 – 6,9 mg/L	12,5 – 16,5 %	20,1 – 25,7 %	ELISA [32]
Casein	Red wines	1,02 mg/L	11,7 %	19,4 %	ELISA [34]
Casein	Red wines	5,6 – 8,5 mg/L	14,7 – 24,0 %	24,8 – 35,6 %	ELISA [34]
Casein	White wines	0,12 -0,80 mg/L	9,1 - 35,0 %	13,7 - 53,8 %	ELISA [34]
Casein	White wines	4,1 – 5,5 mg/L	10,8 – 13,6 %	16,7 – 18,3 %	ELISA [34]
Egg white proteins	Red wines	0,26 mg/L	55,5 %	67,5 %	ELISA [34]
Egg white proteins	Red wines	1,1 – 7,6 mg/L	10,3 – 12,3 %	13,2 – 21,3 %	ELISA [34]
Egg white proteins	White wines	0,59 mg/L	37,4 %	52,1 %	ELISA [34]
Egg white proteins	White wines	3,6 – 6,5 mg/L	11,1 – 17,3 %	17,2 – 22,1 %	ELISA [34]

3.4.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].
Criteria for the level of performance of analytical methods for the quantitative determination of allergens in foods were recently elaborated e.g. by the Ministry of Health and Welfare (MHLW) in Japan [22], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [19-21], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [23] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [18].

Some of the relevant ELISA and PCR validation criteria of the mentioned panels are listed in tables 3 and 4, respectively.

Table 3: ELISA-Validation

Literature [18-24]	**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 an hypothetical proficiency scheme in the range of 0,5 - 5 mg/kg

Table 4: PCR-Validation

Literature [18]	**Recovery rate**	**Repeatability standard deviation**	**Reproducibility standard deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Based on the currently achievable level of performance of ELISA and PCR methods for the quantitative determination of allergens in foods, which could be deduced from the data of precision experiments and from validation criteria, we set a relative target standard deviation σ_{pt} of 25%.
This target standard deviation was applied for the statistical evaluation of the results by z-score or if necessary by z´-Score and was used for all assigned values mentioned in 3.1.

Legal requirements and maximum level recommendations

The labeling of allergens is settled by the regulation of food information for consumers (EU 1169/2011). Especially for wine requirements for labeling of the use of allergen-containing fining agents during winemaking is given in the Implementing Regulation EU 579/2012 [30-33]. Besides sulfite fining agents from milk and egg have to be labeled, if they are detectable in the wine.
Based on data obtained by collaborative studies the International Organisation of Vine and Wine (OIV) settled a limit of detection of ≤ 0,25

mg/L and a limit of quantification of ≤ 0,5 mg/L as criteria for the quantification of casein from milk and albumin and/or lysozyme from egg in wine [33].

3.5 z-Score

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}) [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

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

For information the z-scores below are calculated with a target standard deviation of 25%:

i) ***z-Score*** - z_{ALL} *(with respect to all methods)*
ii) ***z-Score*** - $z_{METHOD\ i}$ *(with respect to single methods)*

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement process, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision, and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (xi) 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 (Ux_{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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

Following the HorRat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.8 Standard uncertainty and metrological traceability

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(Xpt)}$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be

too low with respect to the standard uncertainty of the assigned value.

The metrological traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures

The assigned values and spiking levels are indicated as coloured lines in the figures of results. This allows the comparison of a single result with different possible target values like the spiked level, the robust mean of all results and the robust mean of a single method.

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of allergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.

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 PCR-techniques. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

The following result sections are structured equally for the allergenic components. First all results of ELISA or PCR methods for a certain parameter are reported for samples A and B (qualitative / possibly quantitative) and afterwards for the spiking level sample (quantitative). The recovery rates of results for the spiking level sample and the spiked sample A or B are reported then.

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 skimmed milk powder were converted to **casein**. For this the information supplied in the manufacturer's test kit instructions for the content of casein in skimmed milk powder were taken (ELISA-Systems Test-Kit Manual: 25,6%, Neogen Allergen-Handbuch: 28,8%).

ELISA-Results given as **whole egg powder**, **total egg proteins** (sum egg white and egg yolk proteins) or **ovalbumin** were converted to **egg white proteins**. When possible the information supplied by the test kit manufacturer was used. A content of 26,0 % egg white protein in whole egg powder was taken (Biofront ELISA, Ridascreen ELISA).
Total egg protein was stated for Moringa Kit results. In this case 47% total egg protein in whole egg powder was assumed (source: 46% Nährwerttabellen Souci-Fachmann-Kraut / 48% USDA Nutrient Database).
For ovalbumin a cross-reactivity to egg white proteins of 75% was taken according to test-kit instructions (Immunolab) (corresponding to 75% ovalbumin in egg white proteins).

Results were valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample. Each participant result is valuated qualitatively with respect to the consensus value. The valuation was given as a percentage of results in agreement with the consensus values.

When there are at least 5 quantitative results for all methods or for single methods a statistical evaluation was done.

In cases when a statistical evaluation of the quantitative values was done the result table was given as indicated below:

Evaluation number	Result	Result	z-Score Xpt_{ALL}	z-Score $Xpt_{M\,i}$	Method	Remarks
	pos/neg	[mg/kg]				

The statistical evaluation of results for each parameter was calculated in cases where at least 50% results were positive and at least 5 quantitative values were given:

Characteristics	**All Results** *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{METHOD\ i}}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* σ_{pt}'		
lower limit of target range (X_{pt} - $2\sigma_{pt}$) or (X_{pt} - $2\sigma_{pt}'$)°		
upper limit of target range (X_{pt} + $2\sigma_{pt}'$) or (X_{pt} + $2\sigma_{pt}'$)°		
Quotient S*/σ_{pt} *or* S*/σ_{pt}'		
Standard uncertainty $U_{(Xpt)}$		
Number of results in target range		
Percent in target range		

° Target range is calculated with z-score or z'-score

After that the recovery rates of the results for the spiking level sample and the spiked sample are reported. The number of results within the range of acceptance of 50-150% is given.

4.1 Proficiency Test Milk (Casein)

4.1.1 ELISA-Results: Casein

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
3	positive	>0,1	negative	<0,1	2/2 (100%)	AQ	
4	positive	26,9	negative	<0,2	2/2 (100%)	AQ	
2	positive	14,5	negative	0	2/2 (100%)	BF	
8	positive	>2,6	negative	<0,26	2/2 (100%)	ES	result converted°
6	positive	23,3	negative	<0,1	2/2 (100%)	IL	
5	positive	36,0	negative	<0,25	2/2 (100%)	MI	
1	positive	45,2	negative		2/2 (100%)	RS-F	
7	positive	43,0	negative	<0,25	2/2 (100%)	RS-F	
9	positive	50,7	negative		2/2 (100%)	VT	result converted°

° calculation p. 18

	Sample A		Sample B	
Number positive	9		0	
Number negative	0		9	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus values of results are in qualitative agreement with the spiking of sample A.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Casein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
3	>0,1		AQ	
4	26,9	-0,66	AQ	
2	14,5	-1,8	BF	
8	>2,6		ES	result converted°
6	23,3	-1,0	IL	
5	36,0	0,16	MI	
1	45,2	1,0	RS-F	
7	43,0	0,79	RS-F	
9	50,7	1,5	VT	result converted°

° calculation p. 18

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
A kernel density estimation was not made due to the number of results less than 8.

Characteristics: Quantitative evaluation ELISA Casein

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	7
Number of outliers	0
Mean	34,2
Median	36,0
Robust Mean (X_{pt})	**34,2**
Robust standard deviation (S*)	**14,9**
Target range:	
Target standard deviation $\sigma_{pt'}$	**11,1**
lower limit of target range	**12,1**
upper limit of target range	**56,4**
Quotient S/$\sigma_{pt'}$*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*7,03*
Results in the target range	*7*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The valuation of results was done considering the standard uncertainty of the assigned value by means of z'-score (see 2.1.2 and 3.6).

The evaluation of all methods showed a normal variability of results. The quotient S/$\sigma_{pt'}$ was below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The assigned value Xpt of the evaluation of all results was 49% of the spiking level of casein to sample A and thus at the lower limit of the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Casein" p.27).

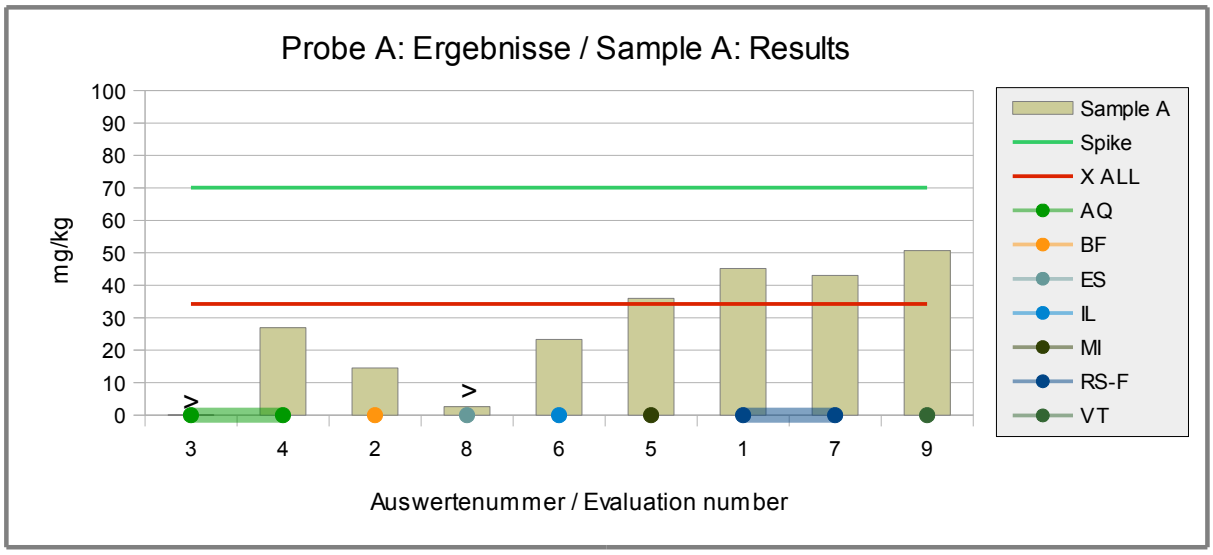


Abb./Fig. 1: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

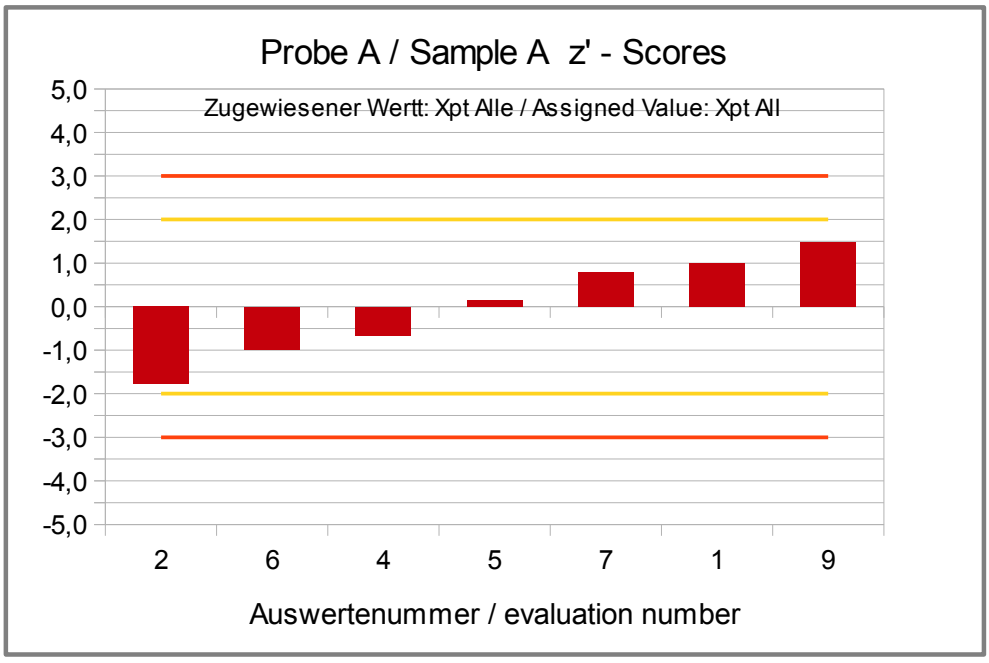


Abb./Fig. 2:
z'-Scores (ELISA Results as Casein)
Assigned value robust mean (algorithm A) of all results

Quantitative evaluation of ELISA results: Spiking level sample

Evaluation number	Casein	z'-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
3	>0,2		AQ	
4	33,0	-1,3	AQ	
2	83,3	1,0	BF	
8	>2,6		ES	result converted°
6	28,1	-1,5	IL	
5	57,0	-0,19	MI	
1	54,0	-0,33	RS-F	
7	63,0	0,09	RS-F	
9	154	4,3	VT	result converted°

° calculation p. 18

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
A kernel density estimation was not made due to the number of results less than 8.

Characteristics: Quantitative evaluation ELISA Casein

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	7
Number of outliers	0
Mean	67,5
Median	57,0
Robust Mean (X_{pt})	**61,0**
Robust standard deviation (S*)	**31,7**
Target range:	
Target standard deviation $\sigma_{pt'}$	**21,4**
lower limit of target range	**18,2**
upper limit of target range	**104**
Quotient S/$\sigma_{pt'}$*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*15,0*
Results in the target range	*6*
Percent in the target range	*86*

Comments to the statistical characteristics and assigned values:

The distribution of results showed a slightly increased variability with a quotient S/σ_{pt} of > 2,0. Therefore the valuation of results was done considering the standard uncertainty of the assigned value by means of z'-score (see 3.6). Then the quotient S*/$\sigma_{pt'}$ was 1,5.*

The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The assigned value Xpt of the evaluation of all results was 73% of the spiking level of casein to the spiking level sample and within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Casein" p.27).

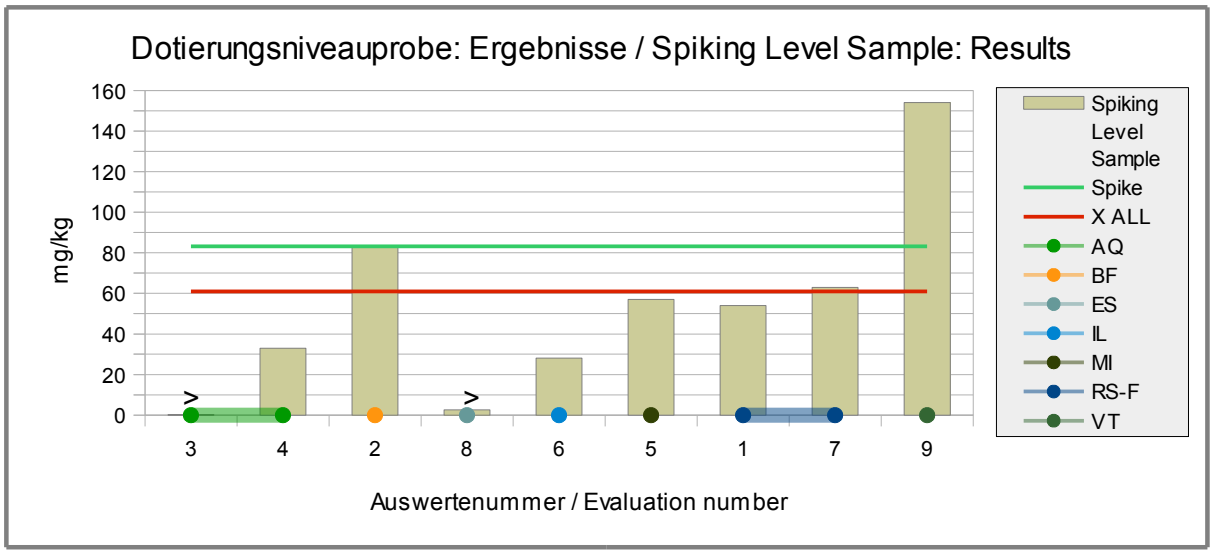


Abb./Fig. 3: ELISA Results Casein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

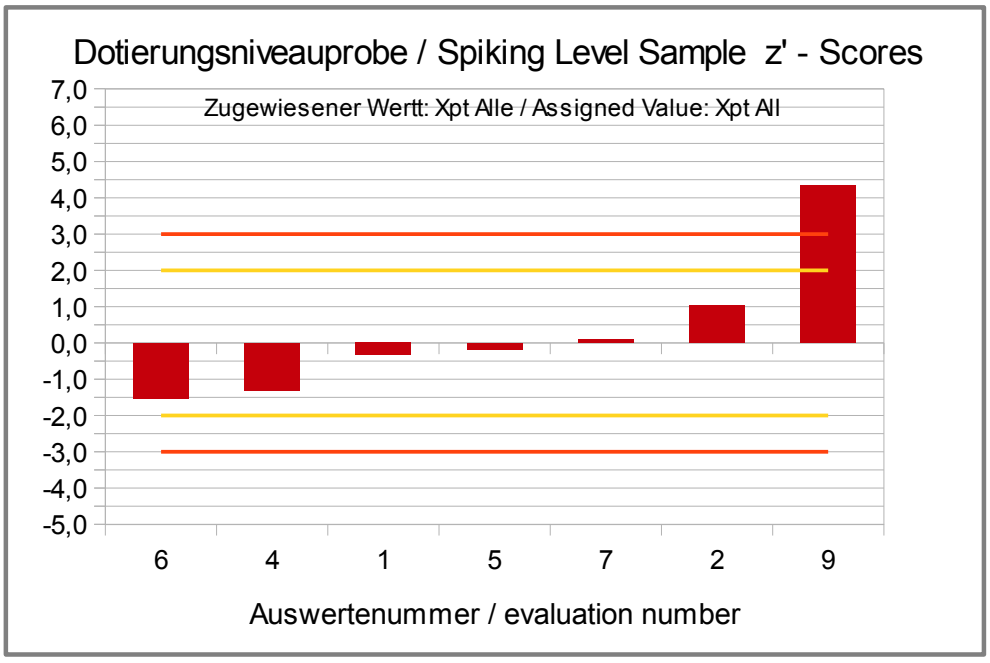


Abb./Fig. 4:
z'-Scores (ELISA Results as Casein)
Assigned value robust mean (algorithm A) of all results

Recovery Rates ELISA for Casein: Spiking level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
3	>0,2		>0,1		AQ	
4	33,0	40	26,9	38	AQ	
2	83,3	**100**	14,5	21	BF	
8	>2,6		>2,6		ES	result converted°
6	28,1	34	23,3	33	IL	
5	57,0	**69**	36,0	**51**	MI	
1	54,0	**65**	45,2	**64**	RS-F	
7	63,0	**76**	43,0	**61**	RS-F	
9	154	185	50,7	**72**	VT	result converted°

° calculation p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	**4**	Anzahl im AB	**4**
Percent in RA	**57**	Prozent im AB	**57**

* Recovery rate 100% relative size: Casein, see p. 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

For the spiking level sample 57% (4) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample sample A also 57% (4) of the recovery rates were in the range of acceptance.

4.2 Proficiency Test Egg (Egg White Proteins)

4.2.1 ELISA-Results: Egg White Proteins, total

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
2	positive	12,4	negative	0	2/2 (100%)	BF	result converted°
4	positive	97,8	negative	<0,05	2/2 (100%)	IL	
6	positive	50,4	negative	<0,04	2/2 (100%)	IL	result converted°
3a	positive	31,7	negative	<0,2	2/2 (100%)	MI	result converted°
5	positive	29,2	negative	<0,2	2/2 (100%)	MI	result converted°
1	positive	28,9	negative		2/2 (100%)	RS-F	result converted°
3b	positive	29,9	negative	<0,07	2/2 (100%)	RS-F	result converted°
7	positive	31,0	negative	< 0,13	2/2 (100%)	RS-F	
8	positive	>3,6	negative	<0,1	2/2 (100%)	RS-F	
9	positive	41,0	negative		2/2 (100%)	VT	

° calculation p. 18

	Sample A		Sample B	
Number positive	10		0	
Number negative	0		10	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
MI = Morinaga Institute ELISA Kit II
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus values of results are in qualitative agreement with the spiking of sample A.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Egg White Proteins	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	12,4	-2,4	BF	result converted°
4	97,8	8,3	IL	outlier excluded
6	50,4	2,3	IL	result converted°
3a	31,7	-0,03	MI	result converted°
5	29,2	-0,34	MI	result converted°
1	28,9	-0,38	RS-F	result converted°
3b	29,9	-0,25	RS-F	result converted°
7	31,0	-0,11	RS-F	
8	>3,6		RS-F	
9	41,0	1,1	VT	

° calculation p. 18

Methods:

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

MI = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

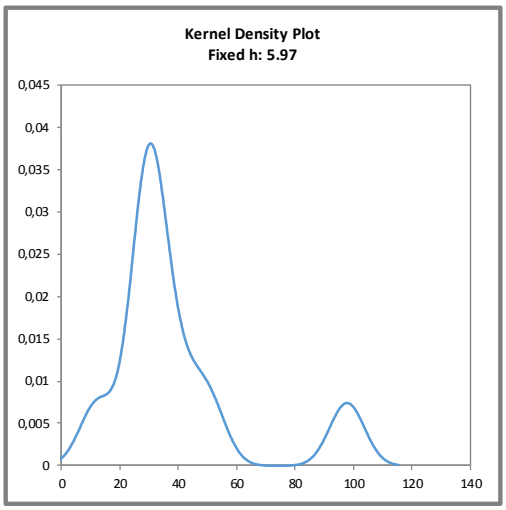


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σ_{pt} of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with two shoulders and a side-peak caused by one result at approx. 100 mg/kg, which was due to a single result (excluded outlier).

Characteristics: Quantitative evaluation ELISA Egg White Proteins

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results°	8
Number of outliers	1
Mean	31,8
Median	30,5
Robust Mean (X)	**31,9**
Robust standard deviation (S*)	**7,96**
Target range:	
Target standard deviation σ_{pt}	**7,98**
lower limit of target range	**16,0**
upper limit of target range	**47,9**
Quotient S/σ_{pt}*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*3,52*
Results in the target range	*6*
Percent in the target range	*75*

° *without result no. 4 (outlier excluded)*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results with two side-peaks caused by two single results. An outlier was excluded from statistical calculations.

The evaluation of all methods showed a normal to low variability of results. The quotient S/σ_{pt} was well below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The assigned values X_{pt} of the evaluation of all results was 60% of the spiking level of egg white proteins to sample A and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Egg White Proteins" p.35).

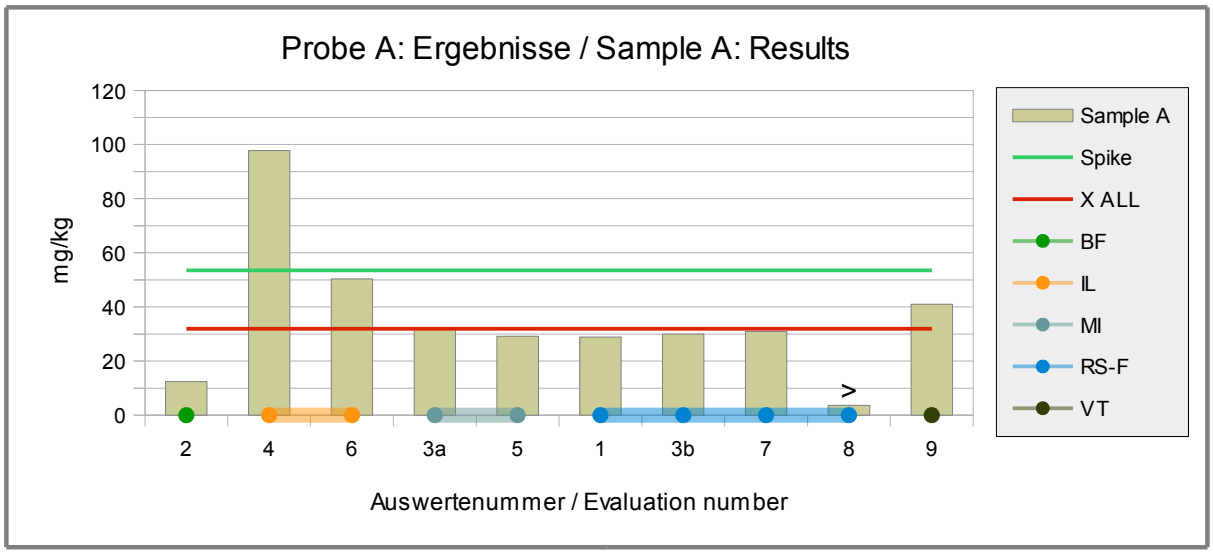


Abb./Fig. 6: ELISA Results Egg White Protein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

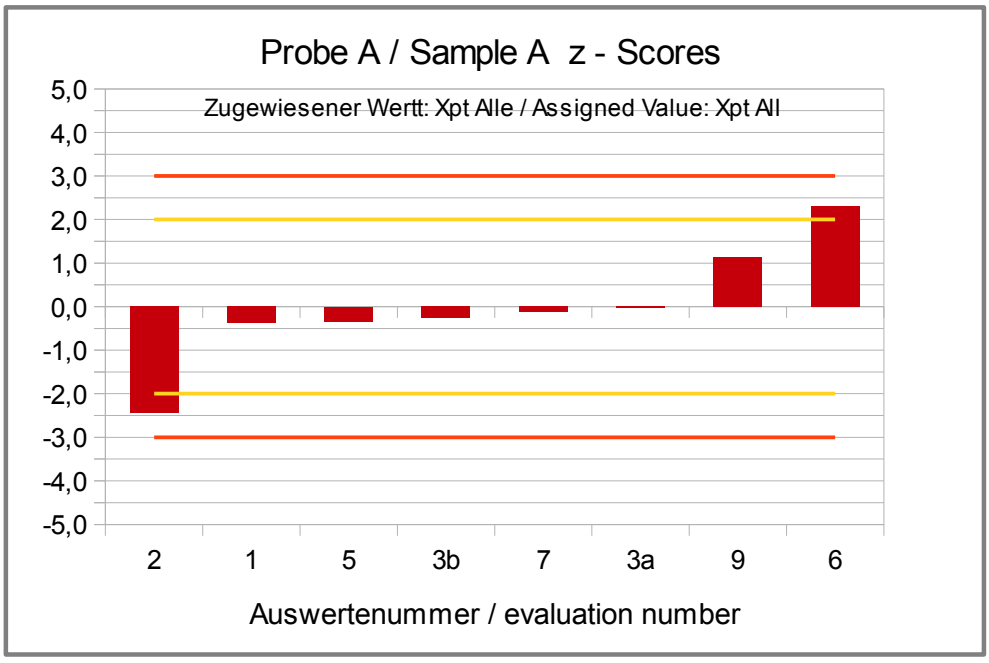


Abb./Fig. 7:
z-Scores (ELISA Results as Egg White Protein)
Assigned value robust mean (algorithm A) of all results

Quantitative evaluation of ELISA results: Spiking level sample

Evaluation number	Egg White Proteins	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
2	28,5	-1,5	BF	result converted°
4	64,7	1,7	IL	
6	46,5	0,08	IL	result converted°
3a	45,0	-0,05	MI	result converted°
5	39,6	-0,53	MI	result converted°
1	46,5	0,08	RS-F	result converted°
3b	34,8	-0,95	RS-F	result converted°
7	43,0	-0,23	RS-F	
8	>3,6		RS-F	
9	62,0	1,4	VT	

° calculation p. 18

Methods:

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

MI = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

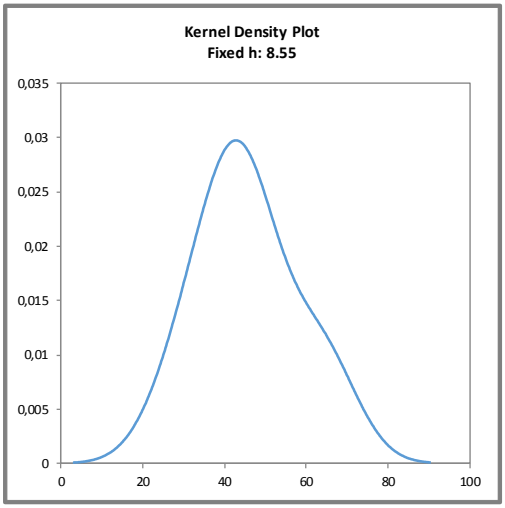


Abb. / Fig. 8:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results.

Characteristics: Quantitative evaluation ELISA Egg White Proteins

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	9
Number of outliers	0
Mean	45,6
Median	45,0
Robust Mean (X)	**45,6**
Robust standard deviation (S*)	**13,2**
Target range:	
Target standard deviation σ_{pt}	**11,4**
lower limit of target range	**22,8**
upper limit of target range	**68,4**
Quotient S/σ_{pt}*	*1,2*
Standard uncertainty $U_{(Xpt)}$	*5,51*
Results in the target range	*9*
Percent in the target range	*100*

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results.

The evaluation of all methods showed a normal to low variability of results. The quotient S/σ_{pt} was well below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The assigned value X_{pt} of the evaluation of all results was 72% of the spiking level of egg white protein to the spiking level sample and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Egg" p.35).

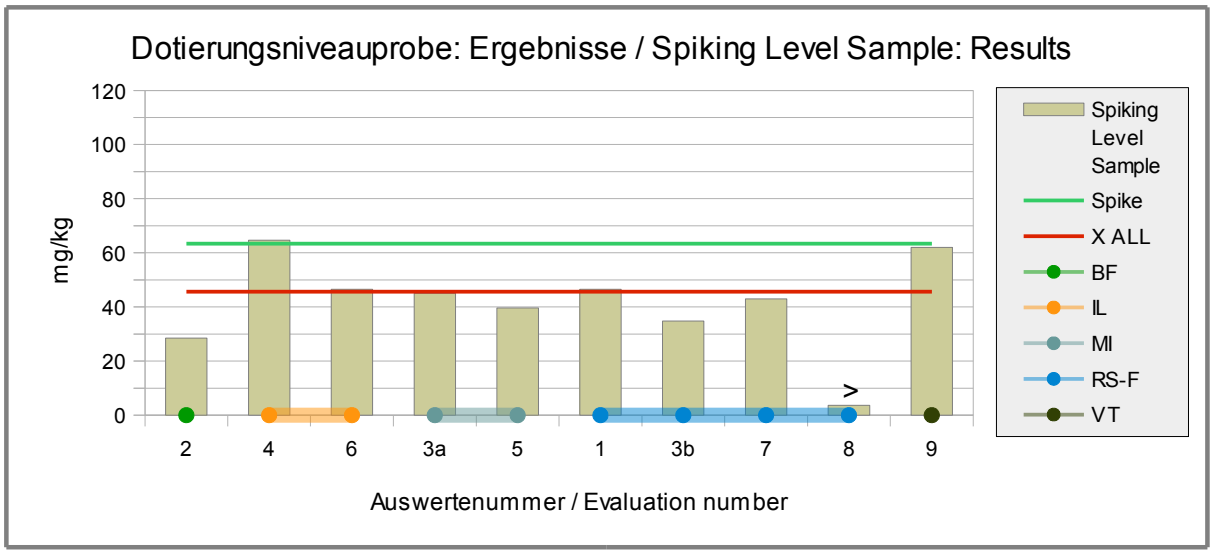


Abb./Fig. 9: ELISA Results Egg White Protein
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

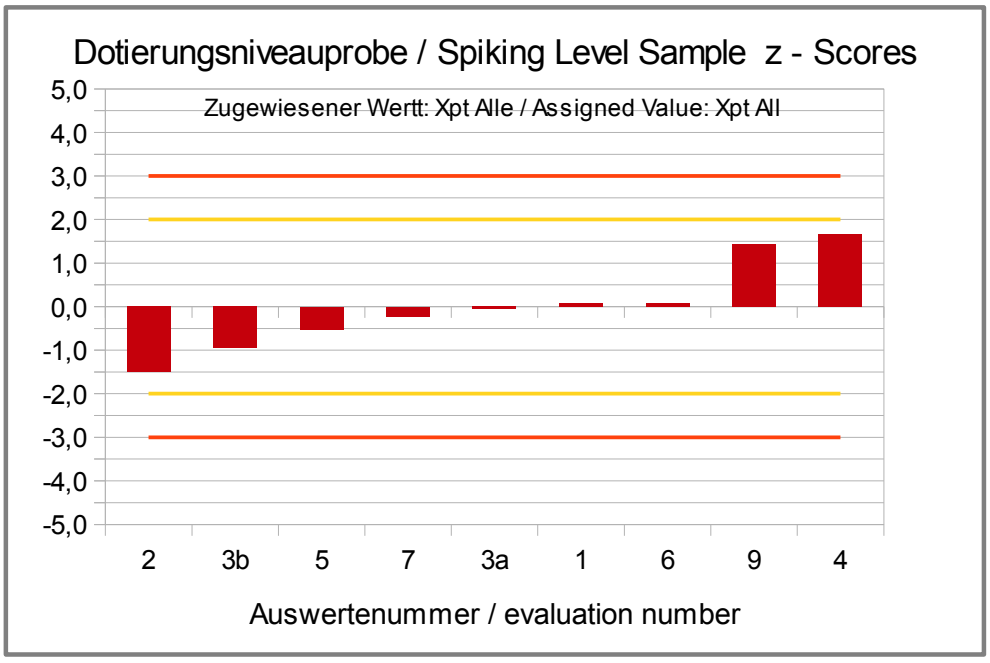


Abb./Fig. 10:
z-Scores (ELISA Results as Egg White Protein)
Assigned value robust mean (algorithm A) of all results

Recovery Rates ELISA for Egg White Proteins: Spiking level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	28,5	45	12,4	23	BF	result converted°
4	64,7	**102**	97,8	183	IL	
6	46,5	**73**	50,4	**94**	IL	result converted°
3a	45,0	**71**	31,7	**59**	MI	result converted°
5	39,6	**62**	29,2	**55**	MI	result converted°
1	46,5	**73**	28,9	**54**	RS-F	result converted°
3b	34,8	**55**	29,9	**56**	RS-F	result converted°
7	43,0	**68**	31,0	**58**	RS-F	
8	>3,6		>3,6		RS-F	
9	62,0	**98**	41,0	**77**	VT	

° calculation p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	**8**	Number in RA	**7**
Percent in RA	**89**	Percent in RA	**78**

* Recovery rate 100% relative size: Egg White Proteins, see p. 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

MI = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

For the spiking level sample 89% (8) of the participants obtained a recovery rate within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample sample A 78% (7) of the recovery rates were in the range of acceptance.

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: Milk (Casein)

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Tag/Monat	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg		Test-Kit + Anbieter
AQ	3	01.03.18	positive	> 0.1	negative	< 0.1	positive	> 0.2	0,2			Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ	4	22.03.18	positive	26,9	negative	<0,2	positive	33	0,2	0,2		Casein	AgraQuant Casein COKAL 1200, RomerLabs
BF	2	12. Apr	positive	14,5	negative	0	positive	83,3	0,12	1		Casein	MonoTrace Milk (Casein) ELISA kit, BioFront Technologies
ES	8		positive	>10	negative	<1	positive	>10		1		Skimmed milk powder	ELISA Systems Casein ESCASPRD-48
IL	6	05.03.18	positive	23,3	negative	< 0.1	positive	28,1	0.04	0.2		Casein	Immunolab Casein ELISA
Mi	5	06.3.	positive	36	negative	<0,25	positive	57	0,25	0,25		Casein	Morinaga Casein ELISA Kit II (M2113)
RS-F	1		positive	45,2	negative		positive	54	0,25	0,25	12,2	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-F	7	07.03.18	-	43	-	< 0,25	-	63	0,12	0,25		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
VT	9	14.03.18	positive	176	negative		positive	534	1			Skimmed milk powder	Veratox Casein Allergen, Neogen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	Meth. Abk.
AQ	3		Application Note Wine, Spiking level sample according to regular instructions	yes	
AQ	4	Casein, more details not known	Spiking level sample pre-diluted with extraction buffer	no	
BF	2	Anti-casein monoclonal	1:10 extraction ratio at 60C for 10 minutes	no	
ES	8			no	
IL	6	Casein			
Mi	5	Casein (Milk protein)	as per kit instructions	yes	
RS-F	1	against Casein		no	
RS-F	7		Extraction with extraction buffer, 60 °C, 10 min	yes	
VT	9			yes	Neogen Total milk

5.1.2 ELISA: Egg (Egg White Proteins)

Meth. Abr.	Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		Tag/Monat	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg		Test-Kit + Anbieter
BF	2	12. Apr	positive	47,7	negative	0	positive	107,2	0,3	1		Whole egg powder	BF
IL	4	22.03.18	positive	97,8	negative	<0,05	positive	64,7	0,5	0,5		Egg white proteins, total	IL
IL	6	05.03.18	positive	37,8	negative	< 0.03	positive	34,9	0,004	0,025		Ovalbumin	IL
MI	3a	08.03.18	positive	57,7	negative	< 0.31	positive	81,9	0,31	0,31		Egg protein	MI
MI	5	09.3.	positive	53	negative	<0,31	positive	72	0,31	0,31		Whole egg protein	MI
RS-F	1		positive	111	negative		positive	178,7	0,25	0,25	17,4	Whole egg powder	RS-F
RS-F	3b	12.03.18	positive	115	negative	< 0.25	positive	134	0,5	0,5		Volleipulver	RS-F
RS-F	7	07.03.18	-	31	-	< 0,13	-	43	0,03	0,13		Egg White Protein	RS-F
RS-F	8		positive	>3,6	negative	<0,1	positive	>3,6	0,03	0,1		Egg white proteins, total	RS-F
VT	9	12.03.18	positive	41	negative		positive	62	1			Egg white proteins, total	VT

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	Meth. Abk.
BF	2	Anti-Ovamucoid monoclonal	1:20 extraction ratio at 60C for 10 minutes	no	For analyzing w ine, 5% non-fat dry milk added to 1X extraction buffer per kit instructions
IL	4	Ovalbumin, more details not know n	Spiking level sample pre-diluted w ith extraction buffer	no	Kit Immunolab Ovalbumin ELISA;
IL	6	Ovalbumin			
MI	3a		Regular Instruction Short Time Extraction Protocol	no	
MI	5	Ovalbumin	as per kit instructions	yes	
RS-F	1	against egg w hite proteins		no	
RS-F	3b		Application Note Wine, Spiking level sample according to regular instructions	yes	
RS-F	7		Extraction w ith extraction buffer, 60 °C, 10 min	yes	
RS-F	8			yes	
VT	9			yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 09-2018 Spiking Level Sample

Weight whole sample	1,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	15,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	46	18,2
2	5,05	44	17,4
3	5,02	49	19,5
4	5,04	47	18,7
5	5,01	48	19,2
6	5,09	41	16,1
7	5,08	46	18,1
8	5,09	45	17,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,8	Particles
Standard deviation	2,71	Particles
χ^2 (CHI-Quadrat)	1,12	
Probability	**99**	%
Recovery rate	120	%

Normal distribution		
Probenanzahl	8	
Mittelwert	18,1	mg/kg
Standardabweichung	1,07	mg/kg
rel. Standardabweichung	5,92	%
Horwitz Standardabweichung	10,3	%
HorRat-Wert	**0,57**	
Wiederfindungsrate	120	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter (1st letter):

PT number	***DLA 09-2018***
PT name	**Allergens IX: Casein and Egg White Protein in Wine with *"Spiking Level Sample"***
Sample matrix (processing)	***Samples A + B:*** *White wine (Grauer Burgunder, German Quality Wine, Baden 2016) and other food additives and allergenic foods (skimmed milk powder, egg white powder)* ***Spiking Level Sample:*** *Glucose, other food additives and allergenic foods (skimmed milk powder, egg white powder)*
Number of samples and sample amount	*2 different Samples A + B: 50 ml each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative:* *Milk (Casein), Egg white protein* *Samples A + B: < 500 mg/kg* *Spiking Level Sample: < 500 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. It is the best to homogenize the whole sample (here by shaking, stirring)*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
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 April 13th 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
		Germany
		USA
		Germany
		Germany
		Germany
		Germany
		Germany
		SPAIN

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

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

7. Index of references

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