Evaluation Report
proficiency test

DLA ptAL09

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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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 14 June 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Content

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 test material of the food matrix samples is a common in commerce rose wine "Cabernet Sauvignon Rosato" (Italy). 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 containing the allergenic ingredients skimmed milk powder and egg white powder (wine treatment agent) were dissolved in the basic mixture and the mixture was homogenized.
The **spiking level sample** was produced with the allergenic compounds above mentioned by 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 DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Rose Wine, organic Labelling: Cabernet Sauvignon Rosato 2018, Italy, contains sulfites,12,0 % vol Pre-treatment: pH adjusted with sodium carbonate solution to pH 7-8	99,7 g/100 g	100 g/100g	-
Glucose	0,27 g/100 g	-	99,96 g/100 g
Milk: Skimmed milk powder mixture (9 products from Europe, USA) - as Skimmed Milk Powder* - *thereof 33,0% total protein*** - *thereof Casein**** - *thereof β-Lactoglobulin****	 243 mg/kg 80,3 mg/kg 64,2 mg/kg 8,0 mg/kg	-	 280 mg/kg 92,4 mg/kg 73,9 mg/kg 9,2 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) - *thereof Lysozyme****	 69,7 mg/kg 53,3 mg/kg 1,87 mg/kg	-	 80,2 mg/kg 61,3 mg/kg 2,15 mg/kg

*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 white protein)
*** Protein calculated according to literature (approx. 80% caseins and approx. 10% β-lactoglobulin in total milk protein [29] and approx. 3,5% lysozyme in egg white protein [30, 35])

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 spiking level sample showed a probability of 54%. 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 1,2. 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, ovalbumin and lysozyme (see next page). The resulting standard deviations between the samples of < 15% were considered sufficient for the applied methods [18, 19, 22, 23].

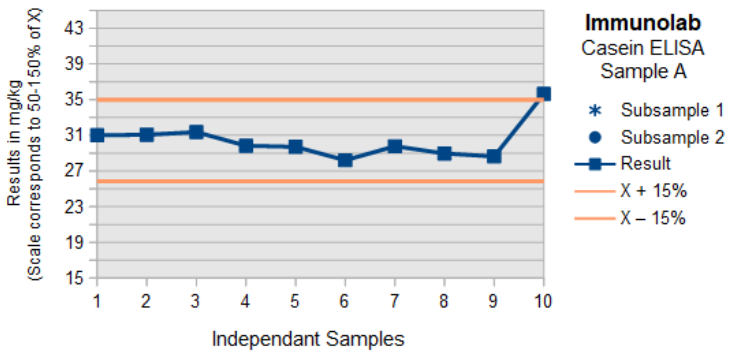
In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.6 and 3.8) [3].

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 30,4 ± 2,1 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg
1	-	-	31,0
2	-	-	31,1
3	-	-	31,4
4	-	-	29,8
5	-	-	29,7
6	-	-	28,2
7	-	-	29,8
8	-	-	29,0
9	-	-	28,6
10	-	-	35,6
	General average X		30,4
	SD of samples		2,12
			7,0%

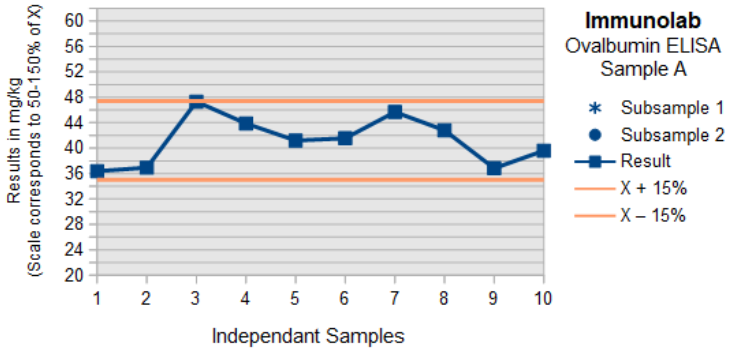


ELISA-Tests: Homogenität Ei / Homogeneity Egg

Immunolab Ovalbumin ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Ovalbumin 41,2 ± 3,8 mg/kg

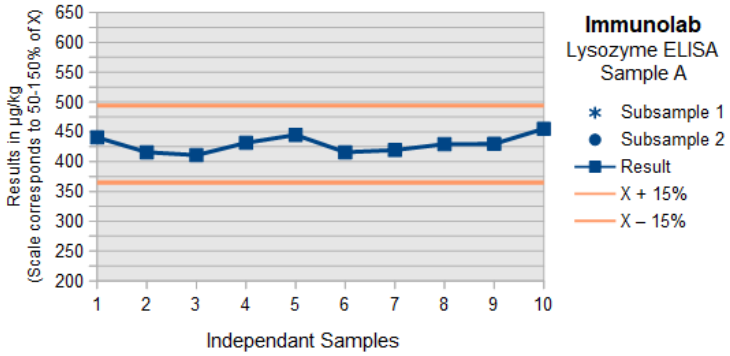
Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg
1	-	-	36,4
2	-	-	36,9
3	-	-	47,3
4	-	-	43,9
5	-	-	41,2
6	-	-	41,6
7	-	-	45,7
8	-	-	42,8
9	-	-	36,8
10	-	-	39,6
	General average X		41,2
	SD of samples		3,81
			9,2%



Immunolab Lysozyme ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Lysozyme 429 ± 14 µg/kg

Sample A	Subsample 1 µg/kg	Subsample 2 µg/kg	Result µg/kg
1	-	-	441
2	-	-	416
3	-	-	411
4	-	-	431
5	-	-	445
6	-	-	416
7	-	-	419
8	-	-	429
9	-	-	430
10	-	-	455
	General average X		429
	SD of samples		14,3
			3,3%



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.

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 spiking level sample was approx. 0,43 (20,4°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 7th week of 2020. The testing method was optional. The tests should be finished at 27th March 2020 the latest (extended).

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 (as **Skimmed Milk Powder, Casein**) and Egg (as **Egg White Protein, Ovalbumin, Lysozyme**) in the range of mg/kg in the matrix of wine (Rosé). 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 and should be analysed like a normal sample.*

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 handed out with the samples (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, limit of quantifications, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 11 participants submitted their results in time.

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 level 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 methods for the quantitative 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*) 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^*_{ALL}***
ii) ***Robust standard deviation of single methods - $S^*_{METHOD\ i}$***
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, too few significant digits (valid digits) or results for 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 procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [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 (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(xpt)}$) [3].

The calculation is performed by:

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

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

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

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 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 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 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 of assigned values

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 llergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.
The corresponding z-scores were calculated according to 3.5 with the target standard deviation of 25% (see 3.4.3).

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**, **total milk protein** and **sum of casein and beta-lactoglobulin** 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 (Neogen Allergen-Handbuch: 28,8%). Results as total milk protein were converted to casein using the literature value of 80% casein in total milk protein. One result that was given as the sum of casein and beta-lactoglobulin was converted to casein using the literature values of 10 % beta-lactoglobulin and 80% casein (AgraQuant milk).

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 26,3% for Ridascreen ELISA and 26% egg white protein in whole egg powder for all others was taken [36].
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) and then converted to egg white protein using the literature value of 26% egg white protein in whole egg powder.
For ovalbumin a cross-reactivity to egg white proteins of 75% was taken according to test-kit instructions (SensiSpec) (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 (Xpt)	$\boldsymbol{Xpt_{ALL}}$	$\boldsymbol{Xpt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (Xpt)		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σpt *or* $\sigma pt'$		
lower limit of target range ($Xpt - 2\sigma pt$) or ($Xpt - 2\sigma pt'$)°		
upper limit of target range ($Xpt + 2\sigma pt$) or ($Xpt + 2\sigma pt'$)°		
Quotient S*/σpt *or* S*/$\sigma pt'$		
Standard uncertainty $U(Xpt)$		
Number of results in target range		
Percent in target range		

° Target range calculated using 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		
4a	positive	30,0	negative	<LOD	2/2 (100%)	AQ-C	
11	positive	1,30	negative	<0,2	2/2 (100%)	AQ-C	
4b	positive	38,4	negative	<LOD	2/2 (100%)	AQ-M	Result converted °
10	positive	49,0	negative		2/2 (100%)	IL	
2	positive	71,0	negative	<0,25	2/2 (100%)	MI-II	
5	positive	89,9	negative		2/2 (100%)	RS-FC	
6	positive	58,0	negative	<0,5	2/2 (100%)	RS-FC	
8a	positive	75,6	negative	<2,5	2/2 (100%)	RS-FC	
9	positive	29,5	negative		2/2 (100%)	RS-FC	
8b	positive	147	negative	<2,0	2/2 (100%)	RS-FM	Result converted °
1	positive	68,5	negative	<0,18	2/2 (100%)	SP	Result converted °
3	positive	44,1	negative	0,12	2/2 (100%)	VT	Result converted °

° calculation see p. 18

	Sample A		Sample B	
Number positive	12		0	
Number negative	0		12	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ-C = AgraQuant Casein, RomerLabs
AQ-M = AgraQuant Milk, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-FC= Ridascreen® Fast Casein, R-Biopharm
RS-FM= Ridascreen® Fast Milk, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative valuation of ELISA: Sample A

Evaluation number	Casein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4a	30,0	-1,8	AQ-C	
11	1,30		AQ-C	Result excluded
4b	38,4	-1,2	AQ-M	Result converted °
10	49,0	-0,46	IL	
2	71,0	1,1	MI-II	
5	89,9	2,5	RS-FC	
6	58,0	0,19	RS-FC	
8a	75,6	1,5	RS-FC	
9	29,5	-1,9	RS-FC	
8b	147		RS-FM	Result converted °, Result excluded
1	68,5	0,94	SP	Result converted °
3	44,1	-0,82	VT	Result converted °

° calculation see p. 18

Methods:

AQ-C = AgraQuant Casein, RomerLabs
AQ-M = AgraQuant Milk, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-FC= Ridascreen® Fast Casein, R-Biopharm
RS-FM= Ridascreen® Fast Milk, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

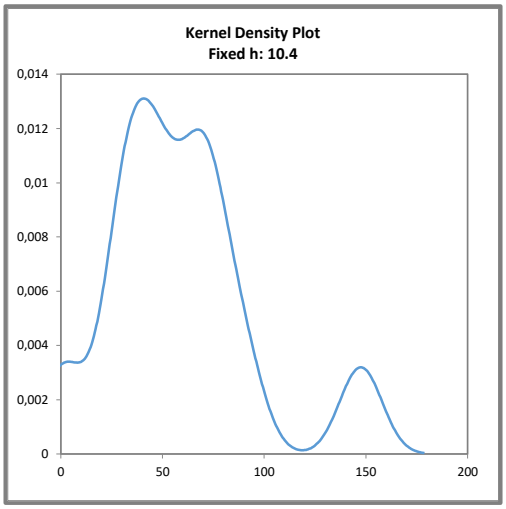


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comments:
The kernel density estimation shows an overlapping, two-peak distribution of results with a shoulder at < 10 mg/kg and a secondary peak at 147 mg/kg, due to two single values outside the target range. A method dependency of the slightly bimodal distribution is not recognizable.

Characteristics: Quantitative evaluation ELISA casein

Sample A

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results°	10
Number of outliers	2
Mean	55,4
Median	53,5
Robust Mean (X_{pt})	**55,4**
Robust standard deviation (S*)	**23,3**
Target range:	
Target standard deviation σ_{pt}	**13,8**
lower limit of target range	**27,7**
upper limit of target range	**83,1**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*9,20*
Results in the target range	*9*
Percent in the target range	*90*

° without results no. 8b and 11 (excluded in advance)

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no method-dependent differences.

The evaluation of all methods showed a normal variability of results, with a quotient S/σ_{pt} below 2,0.*
The robust standard deviation is in the range of established values for the repeatability and 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 robust mean of the evaluation was 86% of the spiking level of casein to sample A and was in the range of the recommendations for the applied methods (s. 3.4.3 and p.27 "Recovery Rates with z-Scores ELISA for Casein").

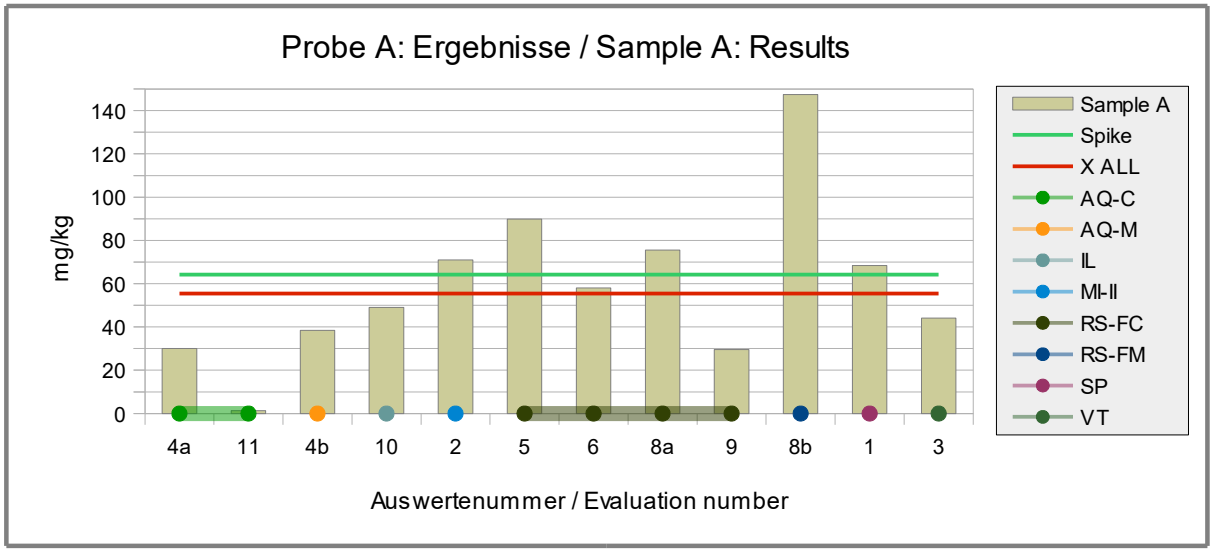


Abb./Fig. 2: ELISA Results casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

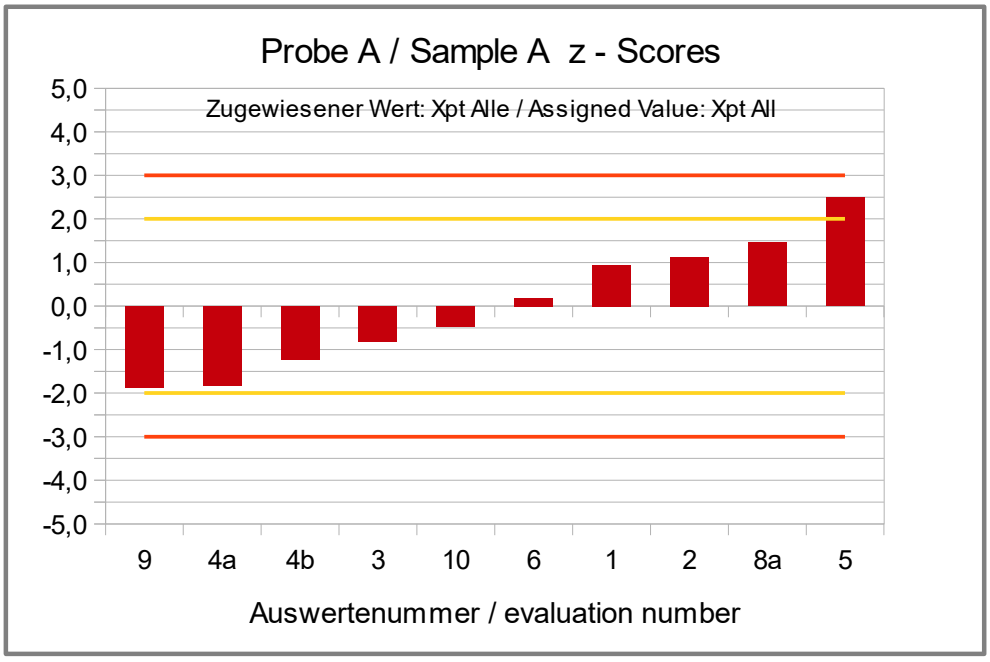


Abb./Fig. 3:
z-Scores ELISA Results as casein
Assigned value robust mean of all results

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Casein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4a	75,0	0,24	AQ-C	
11	46,4	-1,4	AQ-C	
4b	59,2	-0,65	AQ-M	Result converted °
10	71,0	0,01	IL	
2	76,0	0,30	MI-II	
5	152		RS-FC	Result excluded
6	86,7	0,90	RS-FC	
8a	61,1	-0,54	RS-FC	
9			RS-FC	
8b	159		RS-FM	Result converted °, Result excluded
1	80,3	0,54	SP	Result converted °
3	75,7	0,28	VT	Result converted °

° calculation see p. 18

Methods:

AQ-C = AgraQuant Casein, RomerLabs
AQ-M = AgraQuant Milk, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-FC= Ridascreen® Fast Casein, R-Biopharm
RS-FM= Ridascreen® Fast Milk, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

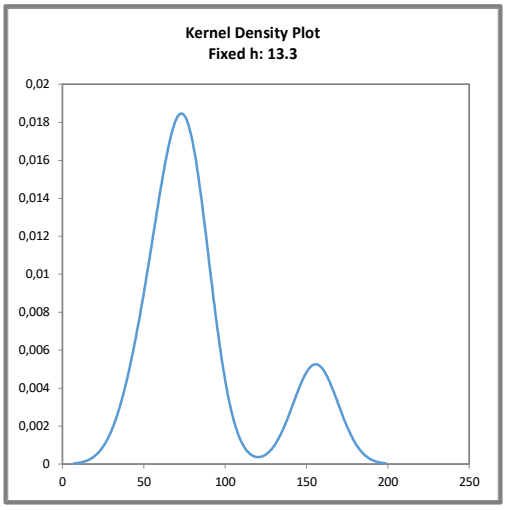


Abb. / Fig. 4:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt_{ALL})

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

Comment:
The kernel density estimation shows a symmetric distribution of results with a secondary peak at approx. 155 mg/kg, due to two single values outside the target range.

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°	9
Number of outliers	2
Mean	70,2
Median	75,0
Robust Mean (X_{pt})	**70,8**
Robust standard deviation (S*)	**12,7**
Target range:	
Target standard deviation σ_{pt}	**17,7**
lower limit of target range	**35,4**
upper limit of target range	**106**
Quotient S/σ_{pt}*	*0,72*
Standard uncertainty $U_{(Xpt)}$	*5,30*
Results in the target range	*9*
Percent in the target range	*100*

° without results no. 5 and 8b (excluded in advance)

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a symmetrical distribution of results (with two high single values).

The evaluation of all methods showed a low variability of results, with a quotient S/σ_{pt} below 1,0.*
The robust standard deviation is in the range of established values for the repeatability and 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 robust mean of the evaluation was 96% of the spiking level of casein to the spiking level sample and was in the range of the recommendations for the applied methods (s. 3.4.3 and p.27 "Recovery Rates with z-Scores ELISA for Casein").

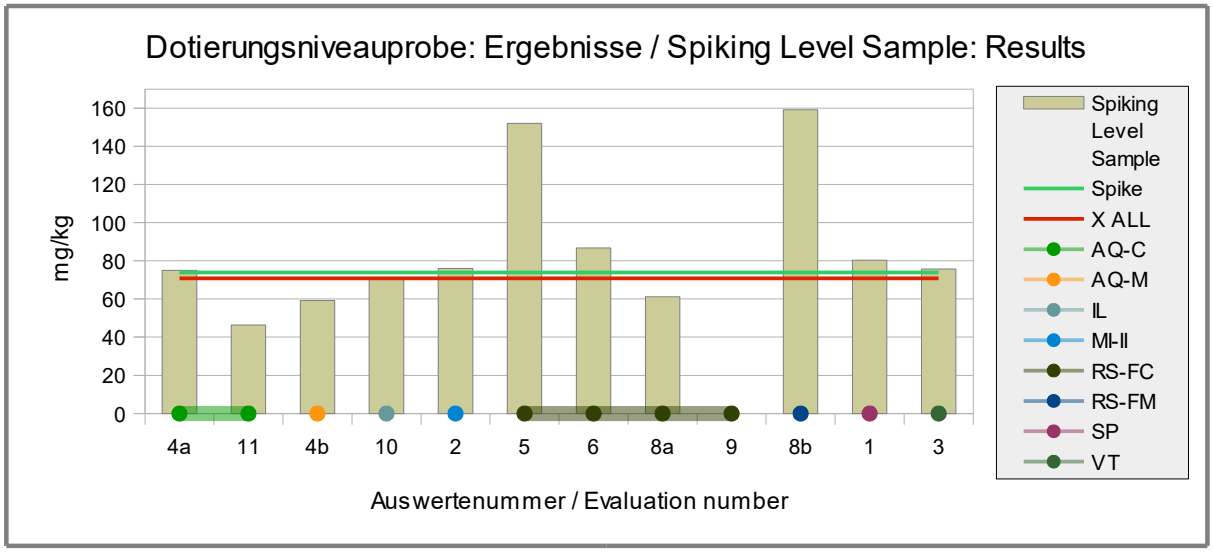


Abb./Fig. 5: ELISA Results casein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

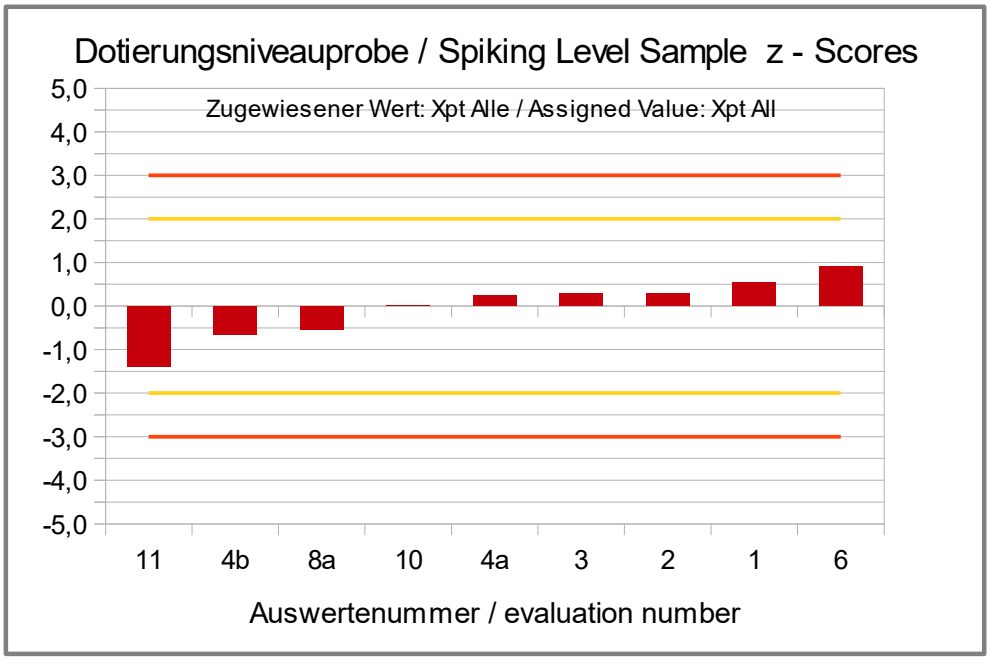


Abb./Fig. 6:
z-Scores ELISA Results as casein
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA for Casein: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
4a	75,0	**101**	0,06	30,0	47	-2,1	AQ-C	
11	46,4	**63**	-1,5	1,30	2	-3,9	AQ-C	
4b	59,2	**80**	-0,80	38,4	**60**	-1,6	AQ-M	Result converted °
10	71,0	**96**	-0,16	49,0	**76**	-0,95	IL	
2	76,0	**103**	0,11	71,0	**111**	0,42	MI-II	
5	152	206	4,2	89,9	**140**	1,6	RS-FC	
6	86,7	**117**	0,69	58,0	**90**	-0,39	RS-FC	
8a	61,1	**83**	-0,69	75,6	**118**	0,71	RS-FC	
9				29,5	46	-2,2	RS-FC	
8b	159	215	4,6	147	230	5,2	RS-FM	Result converted °
1	80,3	**109**	0,35	68,5	**107**	0,26	SP	Result converted °
3	75,7	**102**	0,10	44,1	**69**	-1,3	VT	Result converted °

° calculation see p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	9	Number in RA	8
Percent in RA	82	Percent in RA	67

* Recovery rate 100% relative size: casein, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ-C = AgraQuant Casein, RomerLabs
AQ-M = AgraQuant Milk, RomerLabs
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS-FC= Ridascreen® Fast Casein, R-Biopharm
RS-FM= Ridascreen® Fast Milk, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comment:

82% (9) of the participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 67% (8) of the obtained recovery rates were within the recommended range.
The related z-scores are based on the target standard deviation of 25%.

4.2 Proficiency Test Egg (Egg White Proteins)

4.2.1 ELISA Results: Egg White Proteins

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		
4	positive	23,0	negative	<LOD	2/2 (100%)	AQ	
11	positive	18,2	negative	<0,4	2/2 (100%)	BC	
2	positive	28,2	negative	<0,17	2/2 (100%)	MI	Result converted °
6a	positive	45,4	negative	<0,07	2/2 (100%)	RS	
8	positive	37,3	negative	<0,13	2/2 (100%)	RS	Result converted °
5	positive	34,1	negative		2/2 (100%)	RS-F	
6b	positive	36,9	negative	<0,13	2/2 (100%)	RS-F	
7	positive	13,6	negative		2/2 (100%)	RS-F	
9	positive	24,9	negative		2/2 (100%)	RS-F	Result converted °
10	positive	23,9	negative		2/2 (100%)	RS-F	Result converted °
1	positive	45,6	negative	<0,02	2/2 (100%)	SP	Result converted °
3	positive	39,8	negative	0	2/2 (100%)	VT	Result converted °

° calculation see p. 18

	Sample A		Sample B	
Number positive	12		0	
Number negative	0		12	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
MI = Morinaga Institute ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

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

Quantitative Valuation of ELISA: Sample A

Evaluation number	Egg White Protein	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
4	23,0	-1,0		AQ	
11	18,2	-1,6		BC	
2	28,2	-0,35		MI	Result converted °
6a	45,4	1,9		RS	
8	37,3	0,83		RS	Result converted °
5	34,1	0,41	1,1	RS-F	
6b	36,9	0,77	1,5	RS-F	
7	13,6	-2,2	-2,0	RS-F	
9	24,9	-0,78	-0,27	RS-F	Result converted °
10	23,9	-0,90	-0,41	RS-F	Result converted °
1	45,6	1,9		SP	Result converted °
3	39,8	1,1		VT	Result converted °

° calculation see p. 18

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
MI = Morinaga Institute ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

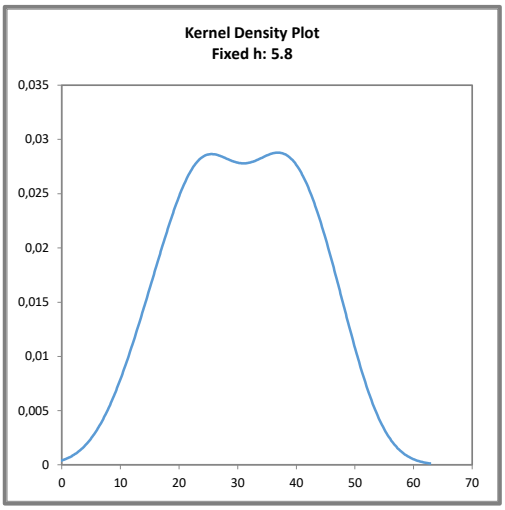


Abb. / Fig. 7:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comment:
The kernel density estimation shows nearly a symmetrical distribution of results with with a slightly bimodal peak. A method dependency cannot be recognized.

Characteristics: Quantitative Evaluation ELISA Egg White Protein

Sample A

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	12	5
Number of outliers	0	0
Mean	30,9	26,7
Median	31,2	24,9
Robust Mean (X_{pt})	**30,9**	**26,7**
Robust standard deviation (S*)	**11,9**	**10,5**
Target range:		
Target standard deviation σ_{pt}	**7,73**	**6,67**
lower limit of target range	**15,5**	**13,3**
upper limit of target range	**46,4**	**40,0**
Quotient S/σ_{pt}*	*1,5*	*1,6*
Standard uncertainty $U_{(X_{pt})}$	*4,29*	*5,85*
Results in the target range	*11*	*5*
Percent in the target range	*92*	*100*

Methoden:
RS-F = R-Biopharm, Ridascreen® Fast

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed a symmetrical distribution of results.

The evaluation of the results of all methods as well as the results of method RS-F showed a normal variability. The quotients S/σ_{pt} were below 2,0. The robust standard deviations were in the range of established values for the repeatability and 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 robust means of the evaluations were 58% and 50% of the spiking level of egg white protein to sample A and were within the range of the recommendations for the applied methods (s. 3.4.3 and p.36 "Recovery Rates with z-Scores ELISA for Egg White Protein").

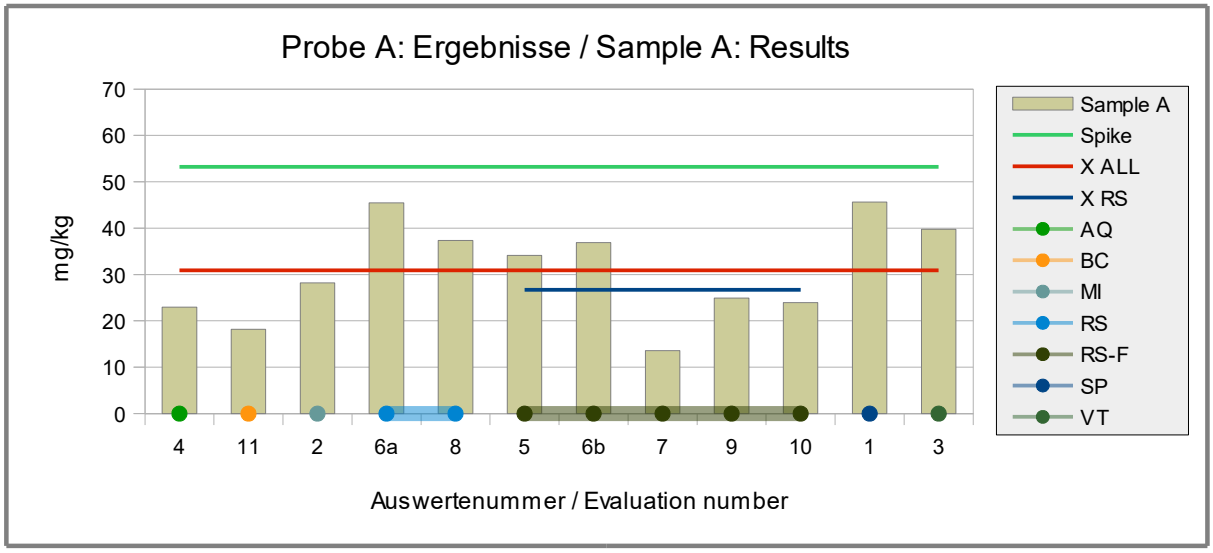


Abb./Fig. 8: ELISA Results egg white protein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-F
round symbols = Applied methods (see legend)

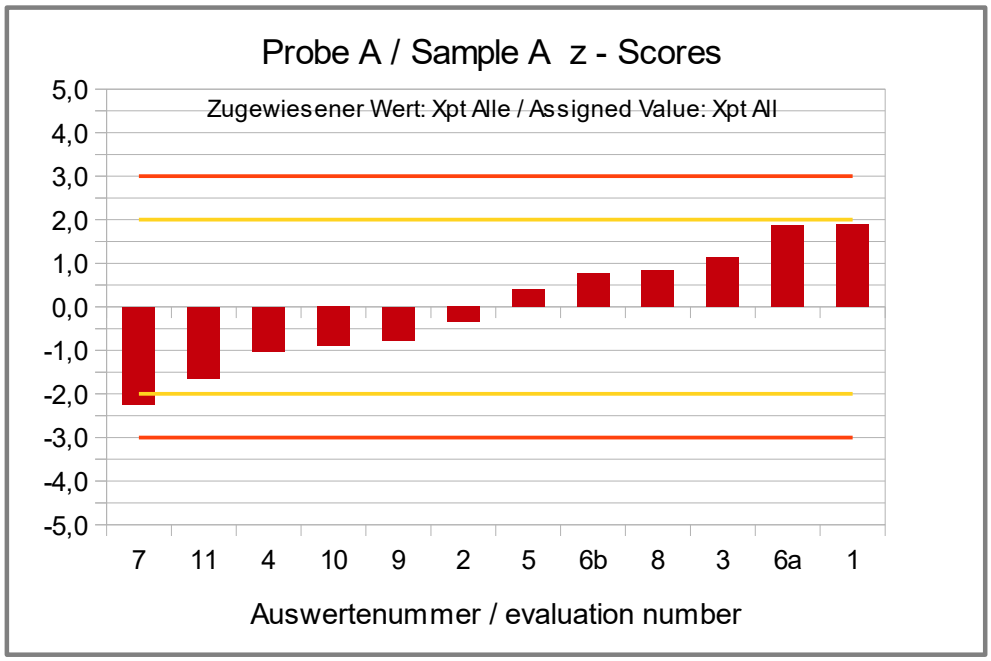


Abb./Fig. 9:
z'-Scores ELISA Results as egg white protein
Assigned value median of all results

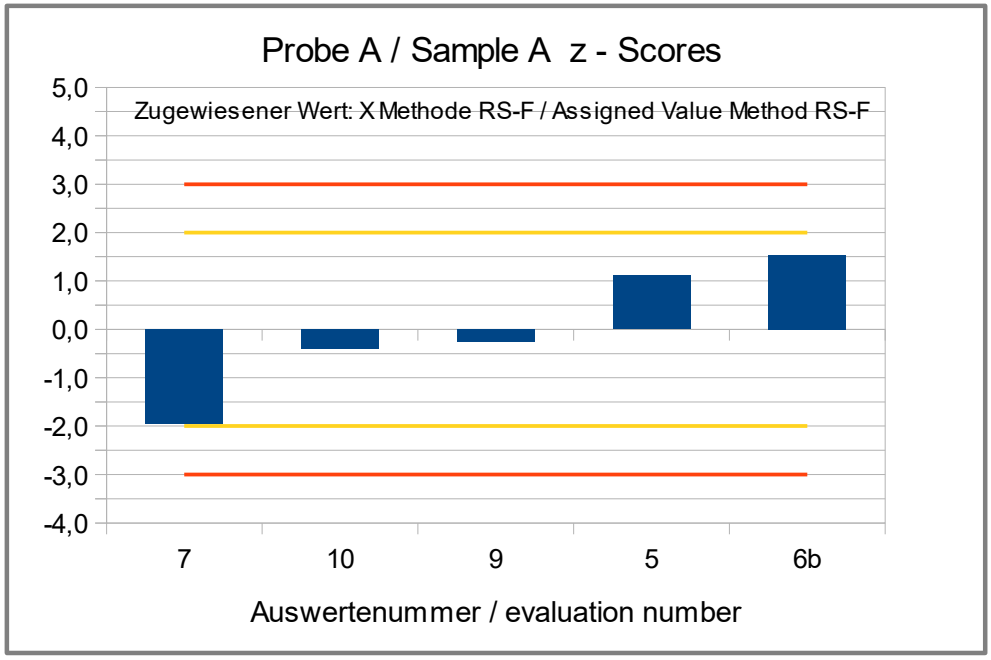


Abb./Fig. 10:
z-Scores ELISA Results as egg white protein, Assigned value robust mean of results method RS-F (R-Biopharm, Ridascreen Fast)

Quantitative Valuation of ELISA: Spiking Level Sample

Evaluation number	Egg White Protein	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]			
4	34,0	-0,71	AQ	
11	33,2	-0,79	BC	
2	29,9	-1,11	MI	Result converted °
6a	59,5	1,8	RS	
8	50,4	0,87	RS	Result converted °
5	38,5	-0,28	RS-F	
6b	47,3	0,57	RS-F	
7	16,5	-2,4	RS-F	
9			RS-F	Result converted °
10	37,3	-0,39	RS-F	Result converted °
1	52,4	1,1	SP	Result converted °
3	51,0	0,92	VT	Result converted °

° calculation see p. 18

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
MI = Morinaga Institute ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

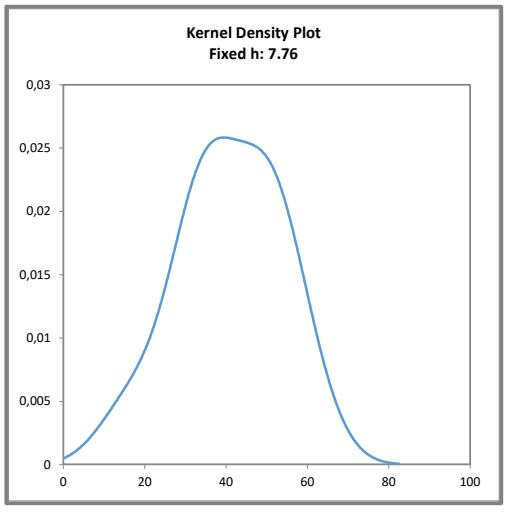


Abb. / Fig. 11:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

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

Characteristics: Quantitative Evaluation ELISA Egg White Protein

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$
Number of results	11
Number of outliers	0
Mean	40,9
Median	38,5
Robust Mean (X_{pt})	**41,4**
Robust standard deviation (S*)	**13,0**
Target range:	
Target standard deviation σ_{pt}	**10,3**
lower limit of target range	**20,7**
upper limit of target range	**62,1**
Quotient S/σ_{pt}*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*4,91*
Results in the target range	*10*
Percent in the target range	*91*

Comments to the statistical characteristics and assigned values:

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

The evaluation of all methods showed a normal variability of results, with a quotient S/σ_{pt} below 2,0.*
The robust standard deviation is in the range of established values for the repeatability and 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 robust mean of the evaluation was 68% of the spiking level of egg white protein to the spiking level sample and were in the range of the recommendations for the applied methods (s. 3.4.3 and p.36 "Recovery Rates with z-Scores ELISA for Egg White Protein").

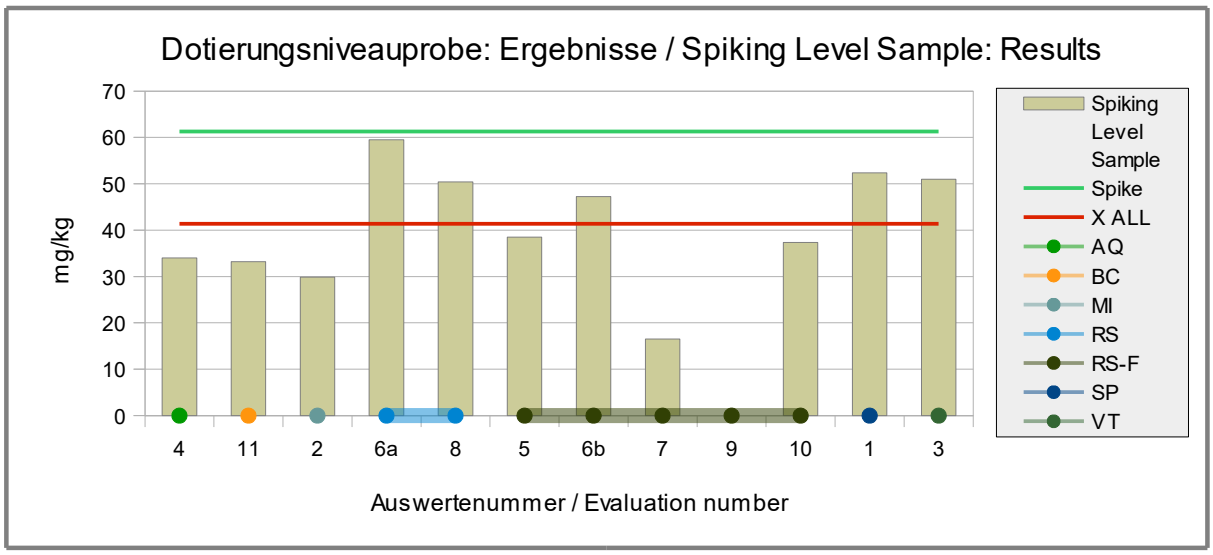


Abb./Fig. 12: ELISA Results Egg White Protein
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

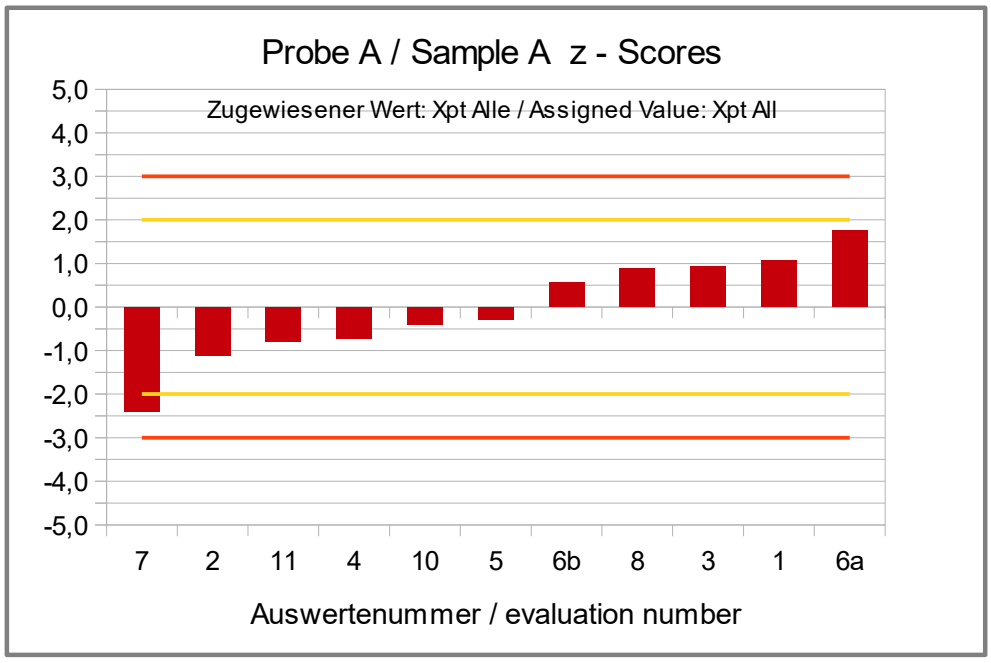


Abb./Fig. 13:
z-Scores ELISA Results as egg white protein
Assigned value robust mean of all results

Recovery Rates with z-Scores ELISA for Egg White Protein: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
4	34,0	**55**	-1,8	23,0	43	-2,3	AQ	
11	33,2	**54**	-1,8	18,2	34	-2,6	BC	
2	29,9	49	-2,0	28,2	**53**	-1,9	MI	Result converted °
6a	59,5	**97**	-0,11	45,4	**85**	-0,59	RS	
8	50,4	**82**	-0,71	37,3	**70**	-1,2	RS	Result converted °
5	38,5	**63**	-1,5	34,1	**64**	-1,4	RS-F	
6b	47,3	**77**	-0,92	36,9	**69**	-1,2	RS-F	
7	16,5	27	-2,9	13,6	26	-3,0	RS-F	
9				24,9	47	-2,1	RS-F	Result converted °
10	37,3	**61**	-1,6	23,9	45	-2,2	RS-F	Result converted °
1	52,4	**85**	-0,58	45,6	**86**	-0,57	SP	Result converted °
3	51,0	**83**	-0,67	39,8	**75**	-1,0	VT	Result converted °

° calculation see p. 18

RA**	50-150 %	RA**	50-150 %
Number in RA	**9**	Number in RA	**7**
Percent in RA	**82**	Percent in RA	**58**

* Recovery rate 100% relative size: egg white protein, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
MI = Morinaga Institute ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen

Comments:

82% (9) participants obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample A 58% (7) recovery rates were in this range of acceptance.
The related z-scores are based on the target standard deviation of 25%.

4.2.2 ELISA Results: Lysozyme

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	0,500	negative	<0,05	2/2 (100%)	RS-F	
6	positive	0,610	negative	<0,05	2/2 (100%)	RS-F	
7	positive	0,470	negative		2/2 (100%)	RS-F	
1	positive	0,430	negative	< 0,013	2/2 (100%)	SP	

	Sample A		Sample B	
Number positive	4		0	
Number negative	0		4	
Percent positive	100		0	
Percent negative	0		100	
Consensus value	positive		negative	

Methods:
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

Quantitative valuation of ELISA: Sample A

No quantitative valuation was done, because there were too few results available. However, all results were within ±50% of the mean of the participant results (0,503 mg/kg).

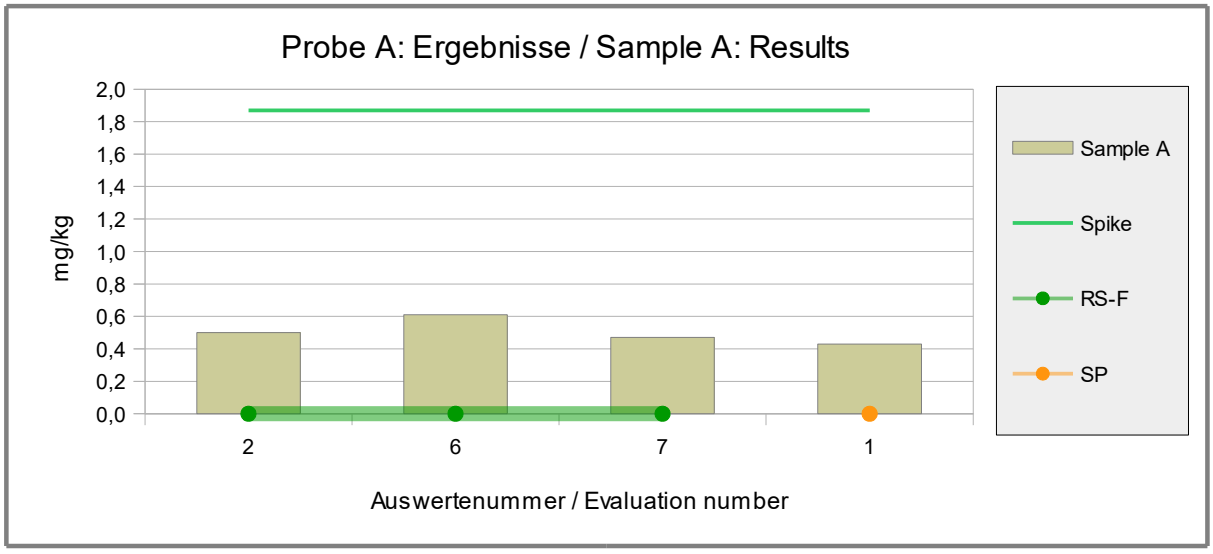


Abb./Fig. 14: ELISA Results Lysozyme
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation of ELISA: Spiking level sample

No quantitative valuation was done, because there were too few results available. However, all results were within ±50% of the mean of the participant results (0,678 mg/kg).

Evaluation number	Lysozyme	Lysozyme	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
2	positive	0,630		RS-F	
6	positive	0,860		RS-F	
7	positive	0,640		RS-F	
1	positive	0,582		SP	

Number positive	4
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

Comment:
For the spiking level sample only positive results were obtained.

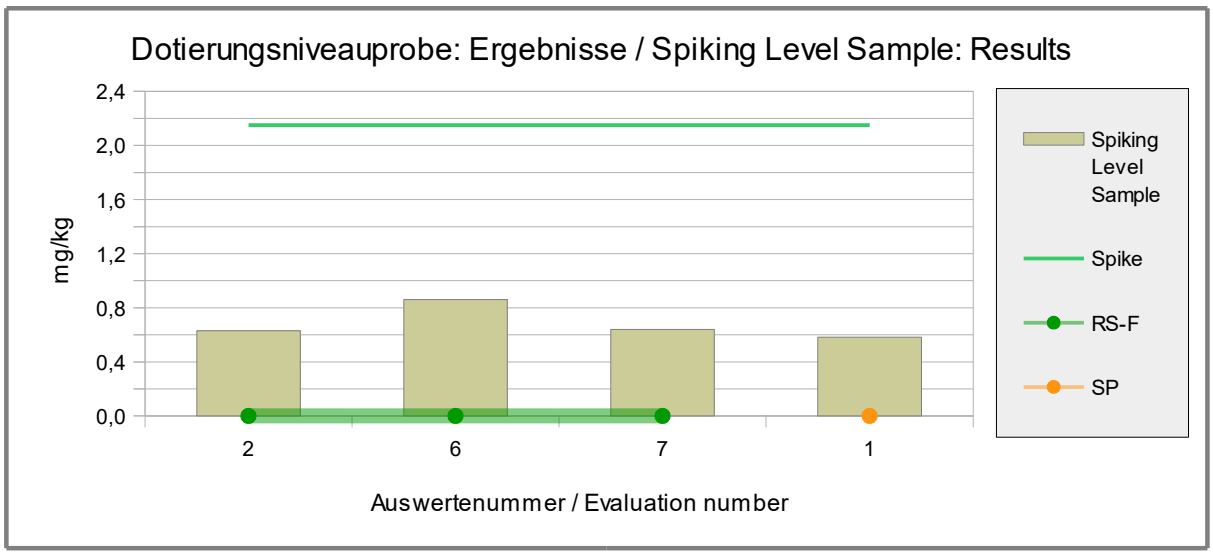


Abb./Fig. 15: ELISA-Results Lysozyme
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates with z-Scores ELISA for Lysozyme: Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*		Sample A	Recovery rate*		Method	Remarks
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]		
2	0,630	29	-2,8	0,500	27	-2,9	RS-F	
6	0,860	40	-2,4	0,610	33	-2,7	RS-F	
7	0,640	30	-2,8	0,470	25	-3,0	RS-F	
1	0,582	27	-2,9	0,430	23	-3,1	SP	

RA**	50-150 %	RA**	50-150 %
Number in RA	0	Number in RA	0
Percent in RA	0	Percent in RA	0

Methods:
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

* Recovery rate 100% relative size: Lysozyme, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Comments:

None of the participants obtained for the spiking level sample or the spiked food matrix sample A a recovery rate by ELISA methods in the range of the AOAC-recommendation of 50-150%. It should be noted that the reference value for lysozyme refers to literature information on the content in the egg white protein (see p. 5).
The related z-scores are based on the target standard deviation of 25%.

4.3 Participant z-Scores: overview table

Z-Scores for the assigned values from participants results (consensus values)

Evaluation number	ELISA Casein: Xpt (div. methods)		ELISA Egg White Protein: Xpt (div. methods)		ELISA Egg White Protein: Xpt (method: RS-F)
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A
1	0,94	0,54	1,9	1,1	-
2	1,1	0,30	-0,35	-1,1	-
3	-0,82	0,28	1,1	0,92	-
4 / 4a	-1,8	0,24	-1,0	-0,71	-
4b	-1,2	-0,65	-	-	-
5	2,5	-	0,41	-0,28	1,1
6 / 6a	0,19	0,90	1,9	1,8	-
6b	-	-	0,77	0,57	1,5
7	-	-	-2,2	-2,4	-2,0
8 / 8a	1,5	-0,54	0,83	0,87	-
8b	-	-	-	-	-
9	-1,9	-	-0,78	-	-0,27
10	-0,46	0,01	-0,90	-0,39	-0,41
11	-	-1,4	-1,6	-0,79	-

Method: RS-F = Ridascreen® Fast, R-Biopharm

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Casein: Xpt (div. methods)		ELISA Egg White Protein: Xpt (div. methods)		ELISA Lysozyme: Xpt (div. methods)	
	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample	Sample A	Sp. Level Sample
1	0,26	0,35	-0,57	-0,58	-3,1	-2,9
2	0,42	0,11	-1,9	-2,0	-2,9	-2,8
3	-1,3	0,10	-1,0	-0,67	-	-
4 / 4a	-2,1	0,06	-2,3	-1,8	-	-
4b	-1,6	-0,80	-	-	-	-
5	1,6	4,2	-1,4	-1,5	-	-
6 / 6a	-0,39	0,69	-0,59	-0,11	-2,7	-2,4
6b	-	-	-1,2	-0,92	-	-
7	-	-	-3,0	-2,9	-3,0	-2,8
8 / 8a	0,71	-0,69	-1,2	-0,71	-	-
8b	5,2	4,6	-	-	-	-
9	-2,2	-	-2,1	-	-	-
10	-0,95	-0,16	-2,2	-1,6	-	-
11	-3,9	-1,5	-2,6	-1,8	-	-

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Casein

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ-C	4a	09.03.20	positive	30	negative	<LOD	positive	75	0,04	0,2	40	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ-C	11	04.05.20	positive	1,3	negative	<0.2	positive	46,4	0,2	0,2	50	Casein	AgraQuant Casein COKAL 1200, RomerLabs
AQ-M	4b	16.03.20	positive	48	negative	<LOD	positive	74	0,05	0,4	50	Milk protein, total	AgraQuant ELISA Milk COKAL2448, RomerLabs
IL	10	28.02.20	positive	49	negative		positive	71	0,05	0,4		Caseinat	Immunolab Milk ELISA
MI-II	2	25.02.	positive	71	negative	<0,25	positive	76	0,25	0,25		Casein	Morinaga Casein ELISA Kit II (M2113)
RS-FC	5	17.03.20	positive	89,9	negative		positive	152	0,5	0,5	25	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-FC	6	12.03.20	positive	57,97	negative	<0,5	positive	86,68	0,12	0,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-FC	8a	26.03.20	-	75,59	-	<2.5	-	61,13	2,5	2,5		Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-FC	9	24.04.20	positive	29,51	negative		-		0,5	0,5	45,22	Casein	Ridascreen® FAST Casein R4612, R-Biopharm
RS-FM	8b	26.03.20	-	184,37	-	<2.5	-	198,94	2,5	2,5		Milk protein, total	Ridascreen® FAST Milk R4652, R-Biopharm
SP	1	18.02.20	positive	77,02	negative	<0.2	positive	90,36				Casein+BLG	SENSISpec Milk ELISA
VT	3	05.03.20	153		0,4		263					Whole Milk Powder	Auswahl Milch-Kits: Neogen

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ-C	4a			yes	
AQ-C	11	Casein	0.5g sample / 10ml extraction Buffer (heated to 60C)	Yes	
AQ-M	4b			yes	
IL	10			yes	
MI-II	2	recognizes cow's milk casein	according to manufacturer's instructions	yes	
RS-FC	5	Casein		yes	
RS-FC	6	Casein	Allergen extraction buffer: 10 min 60°C Allergen extraction buffer: 10 min 60°C	no	
RS-FC	8a	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-FC	9			yes	
RS-FM	8b	As Per Kit Instructions	As Per Kit Instructions	Yes	
SP	1				
VT	3	Antibody	15 min / 60°C	no	

5.1.2 ELISA: Egg White Protein

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	4	20.03.20	positive	23	negative	<LOD	positive	34	0,05	0,4	40	Egg white proteins, total	AgraQuant ELISA Egg White COKAL0848, RomerLabs
BC	11	06.05.20	positive	18,2	negative	<0.4	positive	33,2	0,4	0,4	50	Egg white proteins, total	BioCheck ELISA Egg-Check
MI	2	24.2.	positive	51	negative	<0,31	positive	54	0,31	0,31		Whole egg protein	Morinaga Ei ELISA Kit (M2111)
RS	6a	12.03.20	positive	45,43	negative	<0,07	positive	59,52	0,04	0,07		Egg white proteins, total	Ridascreen® Egg R6411, R-Biopharm
RS	8	26.03.20	-	141,95	-	<0.5	-	191,78	0,5	0,5		Whole egg powder	others: please fill in!
RS-F	5	20.03.20	positive	34,1	negative		positive	38,5	0,5	0,5	21	Egg white proteins, total	Ridascreen® FAST Egg Protein R6402, R-Biopharm
RS-F	6b	12.03.20	positive	36,89	negative	<0,13	positive	47,25	0,03	0,13		Egg white proteins, total	Ridascreen® FAST Ei / Egg R6402, R-Biopharm
RS-F	7	13.03.20	-	13,6	negative		-	16,5	0,03	0,13		Egg white proteins, total	RIDASCREEN FAST Ei , R-Biopharm R6402
RS-F	9	24.02.20	positive	94,74	negative		-		0,5	0,5	31,53	Whole egg powder	other: please enter!
RS-F	10	17.03.20	positive	91	negative		positive	142	0,1	0,5		Whole egg powder	Ridascreen FAST EI/Egg Protein
SP	1	21,02.20	positive	34,22	negative	< 0.013	positive	39,28				Ovalbumin	SENSISpec Ovalbumin ELISA
VT	3	12.03.20	153		0		196					Whole egg powder	Selection Egg-Kits: Neogen

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	4			yes	
BC	11	Ovomucoid	0.5g sample / 10ml extraction Buffer / 15mins / 60C	Yes	
MI	2	recognizes the egg white protein ovalbumin	according to manufacturer's instructions	yes	
RS	6a	Ovalbumin / Ovomukid	Allergene xtraction buffer with skimmed milk powder: 10 min 60°C Allergen extraction buffer sith skimmed milk powder: 10 min 60°C	no	
RS	8	As Per Kit Instructions	As Per Kit Instructions	No	Kit used : Ridascreen® Egg R6411, R-Biopharm
RS-F	5	Egg white proteins		yes	
RS-F	6b	Ovalbumin / Ovomukid	Allergen extraction buffer: 10 min 60°C, Allergen extraction buffer: 10 min 60°C	no	
RS-F	7	Ovalbumin, Ovomucoid from egg white	According to the manufacturer	yes	DT
RS-F	9			yes	Ridascreen FAST Egg R6402
RS-F	10			yes	
SP	1				
VT	3	Antibody	15 min / 60°C	no	

5.1.3 ELISA: Lysozyme

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
RS-F	2	11.03.	positive	0,5	negative	<0,05	positive	0,63	0,2	0,05		Lysozyme	r-biopharm Test-Combination R6452
RS-F	6	12.03.20	positive	0,61	negative	<0,05	positive	0,86	0,006	0,05		Lysozyme	Ridascreen® FAST Lysozym R6452, R-Biopharm
RS-F	7	09.03.20	-	0,47	negative		-	0,64	0,006	0,05		Lysozyme	RIDASCREEN FAST Lysozym, R-Biopharm R6452
SP	1	21.02.20	positive	0,43	negative	< 0.013	positive	0,582				Lysozyme	SENSISpec Lysozyme ELISA

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

Meth. Abbr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
RS-F	2	recognizes lysozyme	according to manufacturer's instructions	yes	
RS-F	6	Lysozyme	Allergen extraction buffer with gelatin: 10 min 60°C Allergen extraction buffer with gelatin: 10 min 60°C	no	
RS-F	7	Chicken egg lysozymes	According to the manufacturer	yes	JGE
SP	1				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptA09 2020 Spiking Level Sample

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,29	57	21,6
2	5,08	56	22,0
3	5,08	74	29,1
4	5,22	67	25,7
5	5,28	67	25,4
6	5,11	54	21,1
7	4,97	64	25,8
8	5,19	57	22,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	62,0	Particles
Standard deviation	7,30	Particles
χ^2 (CHI-Quadrat)	6,02	
Probability	**54**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	24,1	mg/kg
Standard deviation	2,83	mg/kg
rel. Standard deviaton	11,8	%
Horwitz standard deviation	9,9	%
HorRat-value	**1,2**	
Recovery rate	107	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***ptAL09 - 2020***
PT name	***Allergens IX: Milk (Casein) and Egg White Protein in Wine***
Sample matrix (processing)	***Samples A + B:*** *Rosé wine, organic (vegan) / Cabernet Sauvignon Rosato (Italia) 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: 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 (Ovalbumin, Lysozyme)* *Samples A + B: < 500 mg/kg (Lysozyme < 5 mg/kg)* *Spiking Level Sample: < 500 mg/kg (Lysozyme < 5 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***
Last Deadline	***the latest March 27th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		SWITZERLAND
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN
		Germany
		AUSTRIA

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of

a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. Allergen Data Collection - Update (2002): Cow's Milk (Bos domesticus), Besler M., Eigenmann P., Schwartz R., Internet Symposium on Food Allergens 4(1): 19-106, http://www.food-allergens.de
30. Peñas et al. (2015) Allergenic Proteins in Enology: A Review on Technological Applications and Safety Aspects, Molecules 2015, 20, 13144-13164
31. Restani et al. (2012) Validation by a Collaborative Interlaboratory Study of an ELISA Method for the Detection of Caseinate Used as a Fining Agent in Wine, Food Anal. Methods (2012) 5:480–486
32. Restani et al. (2014) Collaborative Interlaboratory Studies for the Validation of ELISA Methods for the Detection of Allergenic Fining Agents Used in Wine According to the Criteria of OIV Resolution 427–2010 Modified by OIV–Comex 502–2012, Food Anal. Methods (2014) 7:706–712
33. RESOLUTION OIV/OENO 427/2010 + 502-2012: CRITERIA FOR THE METHODS OF QUANTIFICATION OF POTENTIALLY ALLERGENIC RESIDUES OF FINING AGENT PROTEINS IN WINE, International Organisation of Vine and Wine 2010 / 2012
34. Lacorn et al. (2014) Collaborative Tests of ELISA Methods for the Determination of Egg White Protein and Caseins Used as Fining Agents in Red and White Wines, Food Anal. Methods (2014) 7:417–429
35. Allergen Data Collection - Update (2000): Hen's Egg White (Gallus domesticus), Barkholt V., Besler M., Sampson H.A., Internet Symposium on Food Allergens 2 (Suppl.1): 1-29, http://www.food-allergens.de
36. Belitz H.D., Grosch W., Schieberle P., Lehrbuch der Lebensmittelchemie, 6. Aufl., Springer-Verlag, Berlin 2008 [Textbook of food chemistry, 6th edition]