Evaluation Report
proficiency test

DLA ptAL10 (2020)

Allergens X:

Gluten

in “gluten-free” Beer

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

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Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s)
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 different PT-samples with the same food matrix were provided for the detection and quantitative determination of the allergens in the range of mg/kg.

The test material is common in commerce "gluten-free" beer (sample A) and common in commerce Pilsener beer (sample B) (see table 1). The respective samples were homogenized by swirling on the shaker.

The samples A and B were portioned to approximately 50 ml in PE bottles with screw lock.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Organic Pilsener Beer (Lager), gluten free Labelling: 4,7%vol alcohol, 11,5% original wort Ingredients: Natural mineral water, barley malt, hops Preservative: potassium sorbate*	100 g/100 g	-
Organic Pilsener Beer (Lager) Labelling: 4,7%vol alcohol, 11,5% original wort Ingredients: Natural mineral water, barley malt, hops Preservative: potassium sorbate*	-	100 g/100 g

*Preservation of the PT samples by DLA

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 **homogeneity of the bottled DLA samples** (sample B containing gluten) was tested by ELISA for the contents of gluten. The resulting standard deviation between the samples of < 15% was considered sufficient for the applied method [18, 19, 22, 23].

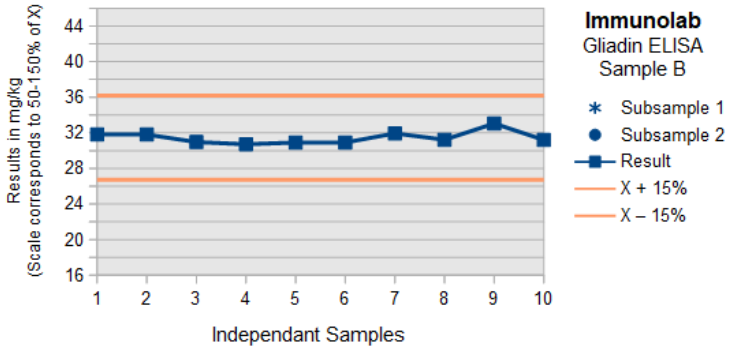
In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.6 and 3.8) [3].

ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin/Gluten ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gliadin 31,5 ± 0,7 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg	
1	-	-	31,8	
2	-	-	31,8	
3	-	-	31,0	
4	-	-	30,7	
5	-	-	30,9	
6	-	-	30,9	
7	-	-	31,9	
8	-	-	31,2	
9	-	-	33,0	
10	-	-	31,2	
	General average X		31,5	
	SD of samples		0,708	2,3%



Note: The reactivity to barley is 5%, therefore the results for the indication as gliadin were multiplied by a factor of 20 (cf. Test Kit Manual, Immunolab).

2.1.2 Stability

The food matrix sample material is beer. In long-term stability tests over one year, the parameter gluten has proven to be stable. Thus the stability of the samples was given under the specified storage conditions during the analysis period.

2.2 Sample shipment and information to the test

The portions of test materials sample A and B were sent to every participating laboratory in the 14th week of 2020. The testing method was optional. The tests should be finished at 12th June 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 with possible levels of gluten from barley malt (from Pilsner beer) in the mg/kg range in the matrix "gluten-free" beer.

Note: Please cool samples on arrival (2 – 10 ° C)

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

2.3 Submission of results

The participants submitted their results in standard forms, which have been 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.

16 of 17 participants submitted their results in time. One participant submitted no results.

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.

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 Working Group on Prolamin Analysis and Toxicity (WGPAT) coordinated a collaborative study with two commercial ELISA test kits for the determination of gluten using the monoclonal R5 antibody [24]. 12 food samples with gliadin in the range of 0 - 168 mg/kg were analyzed by 20 laboratories. Recovery rates ranged between 65 and 110%, relative repeatability deviations ranged from 13 - 25% (method 1) and 11 - 22% (method 2) while the relative reproducibility standard deviations ranged from 23 - 47% (method 1) and 25 - 33% (method 2). According to the authors both ELISA test kits fulfilled therefore the current validation criteria for ELISA methods [24].

The precision data in table 2 were obtained in collaborative trials by a commercial ELISA testkit for determination of gluten in fermented cereal products (AOAC method AACCI 38-55.02) [29]. "Gluten-free" beers made from sorghum and sorghum beers spiked with hordein digest (barley) were studied.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments [29]

Parameter	**Matrix**	**Mean**	**RSD_r**	**RSD_R**	**Method / Literature**
Gluten	"gluten-free" Beer (sorghum beer)	2,36 mg/kg	98,0 %	126,1 %	ELISA [29]
Gluten	"gluten-free" Beer (sorghum beer), spiked	26,2 mg/kg	30,2 %	36,8 %	ELISA [29]
Gluten	"gluten-free" Beer (sorghum beer), spiked	119,5 mg/kg	31,2 %	31,2 %	ELISA [29]
Gluten	"gluten-free" Starch syrup	1,29 mg/kg	157,3 %	236,1 %	ELISA [29]
Gluten	Starch syrup	10,6 mg/kg	16,3 %	34,4 %	ELISA [29]
Gluten	Sourdough	48,4 mg/kg	23,1 %	25,9 %	ELISA [29]
Gluten	Sourdough	145,6 mg/kg	19,5 %	27,5 %	ELISA [29]

In particular, the gluten content can be evaluated differently in fermented cereal products by different ELISA methods: A comparative study of 5 sandwich ELISA and 2 competitive ELISA methods for the determination of gluten in various stages of beer production was performed by Panda et al. (2015) [30].

Colgrave et al. (2014) applied a LC-MS/MS method for the determination of gluten present in hydrolysed form in beer in comparison to ELISA methods [31].

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). For labeling of gluten and gluten containing cereals EU-regulation 828/2014 recommends: Foods with a gluten content of <20 mg/kg may indicated as "gluten-free" and with a content not exceeding 100 mg/kg as "very low gluten".

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.

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 methods for a certain parameter are reported for samples A and B (qualitative/ possibly quantitative) and afterwards for PCR methods.

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

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

In the present PT all ELISA results were submitted uniformly as gluten, therefore no conversion was necessary.

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

4.1 Proficiency Test Gluten

4.1.1 ELISA Results: Gluten

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		
15	negative	0	positive	108	2/2 (100%)	BF	
6	positive	10	positive	64,0	1/2 (50%)	IL	
1	negative	<10	positive	60,0	2/2 (100%)	RS-C	
2	positive	10	positive	77,3	1/2 (50%)	RS-C	Result sample A at LOQ
3	negative	< 10	positive	69,5	2/2 (100%)	RS-C	
4	negative	<5	positive	72,1	2/2 (100%)	RS-C	
5	negative	<10	positive	51,0	2/2 (100%)	RS-C	
7	negative		positive	60,8	2/2 (100%)	RS-C	
8	positive	19,7	positive	69,2	1/2 (50%)	RS-C	
10	negative	< 10	positive	69,5	2/2 (100%)	RS-C	
11	negative	<10	positive	64,7	2/2 (100%)	RS-C	
12	negative		positive	44,7	2/2 (100%)	RS-C	Mean calculated by DLA
13	negative		positive	64,6	2/2 (100%)	RS-C	
14	negative	<10	positive	123	2/2 (100%)	RS-C	
9	negative	<5	positive	21,0	2/2 (100%)	SP	
16	negative	0,8	positive	15,9	2/2 (100%)	VT-R5	

	Sample A		Sample B	
Number positive	3		16	
Number negative	13		0	
Percent positive	19		100	
Percent negative	81		0	
Consensus value	negative		positive	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-C = Ridascreen® competitive, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT-R5 = Veratox, Neogen

Comments:

The consensus values are in qualitative agreement with the spiking of sample B.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Sample B	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}C}$	Method	Remarks
	[mg/kg]				
15	108	2,8		BF	
6	64,0	0,00		IL	
1	60,0	-0,25	-0,27	RS-C	
2	77,3	0,83	0,80	RS-C	Result sample A at LOQ
3	69,5	0,35	0,32	RS-C	
4	72,1	0,51	0,48	RS-C	
5	51,0	-0,81	-0,83	RS-C	
7	60,8	-0,20	-0,23	RS-C	
8	69,2	0,33	0,30	RS-C	
10	69,5	0,35	0,32	RS-C	
11	64,7	0,05	0,02	RS-C	
12	44,7	-1,2	-1,2	RS-C	Mean calculated by DLA
13	64,6	0,04	0,01	RS-C	
14	123	3,7		RS-C	Outlier excluded for $Xpt_{RS\text{-}C}$
9	21,0	-2,7		SP	
16	15,9	-3,0		VT-R5	

Methods:
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-C = Ridascreen® competitive, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT-R5 = Veratox, Neogen

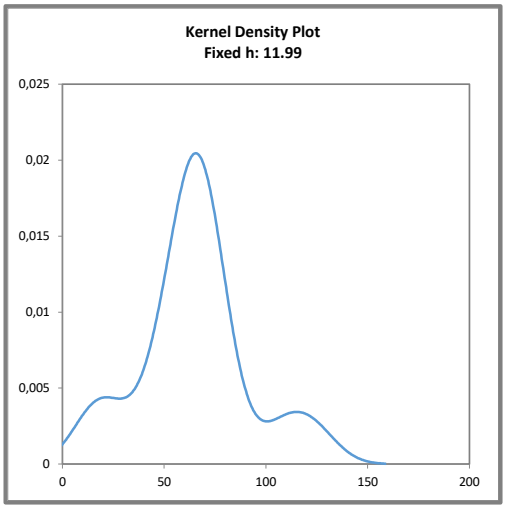


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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 with two secondary peaks at <30 mg/kg and >100 mg/kg, due to single values of different methods.

Characteristics: Quantitative evaluation ELISA Gluten

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-C** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}C}}$
Number of results	16	11°
Number of outliers	-	1
Mean	64,7	64,0
Median	64,6	64,7
Robust Mean (X_{pt})	**64,0**	**64,4**
Robust standard deviation (S*)	**17,6**	**9,63**
Target range:		
Target standard deviation σ_{pt}	**16,0**	**16,1**
lower limit of target range	**32,0**	**32,2**
upper limit of target range	**95,9**	**96,6**
Quotient S/σ_{pt}*	*1,1*	*0,60*
Standard uncertainty $U_{(Xpt)}$	*5,49*	*3,63*
Results in the target range	*12*	*11*
Percent in the target range	*75*	*100*

° *without result no. 14 (excluded in advance)*

Methods:
RS-C = R-Biopharm, Ridascreen® competitive

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed nearly a symmetrical distribution.

The evaluation of the results of all methods as well as the results of method RS-C showed a normal to low variability of results. The quotients S/σ_{pt} were below 2,0. The robust standard deviations were in the lower 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.

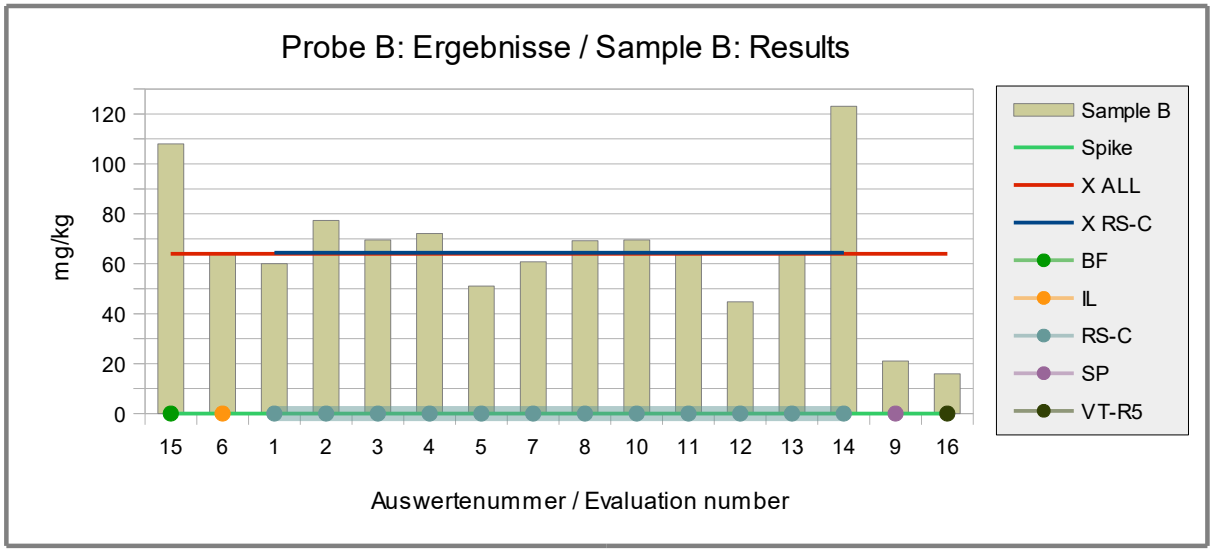


Abb./Fig. 2: ELISA Results Gluten
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-C
round symbols = Applied methods (see legend)

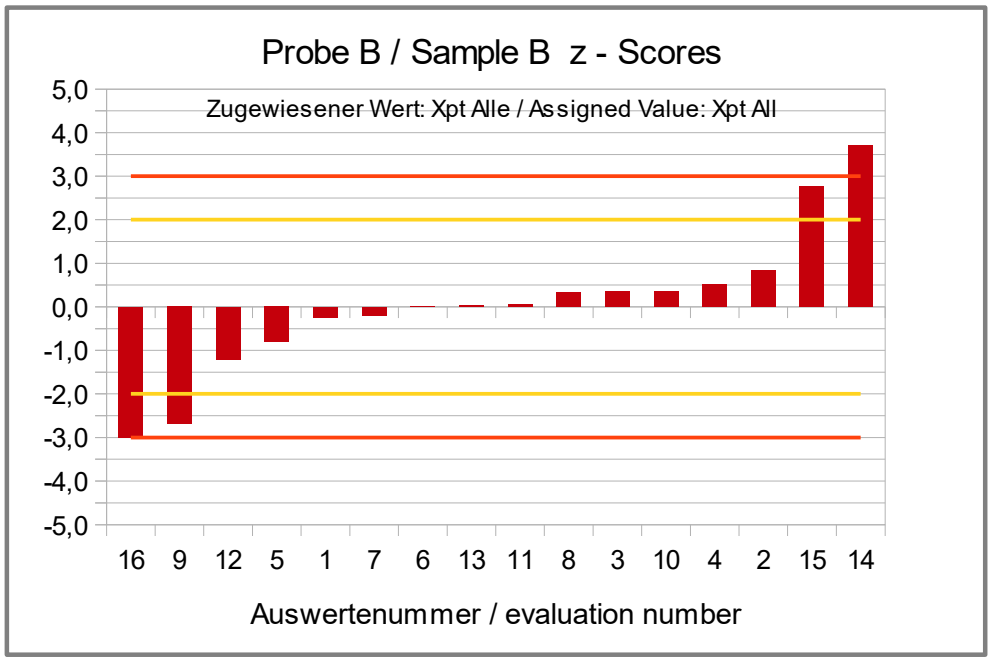


Abb./Fig. 3:
z-Scores ELISA Results Gluten
Assigned value robust mean of all results

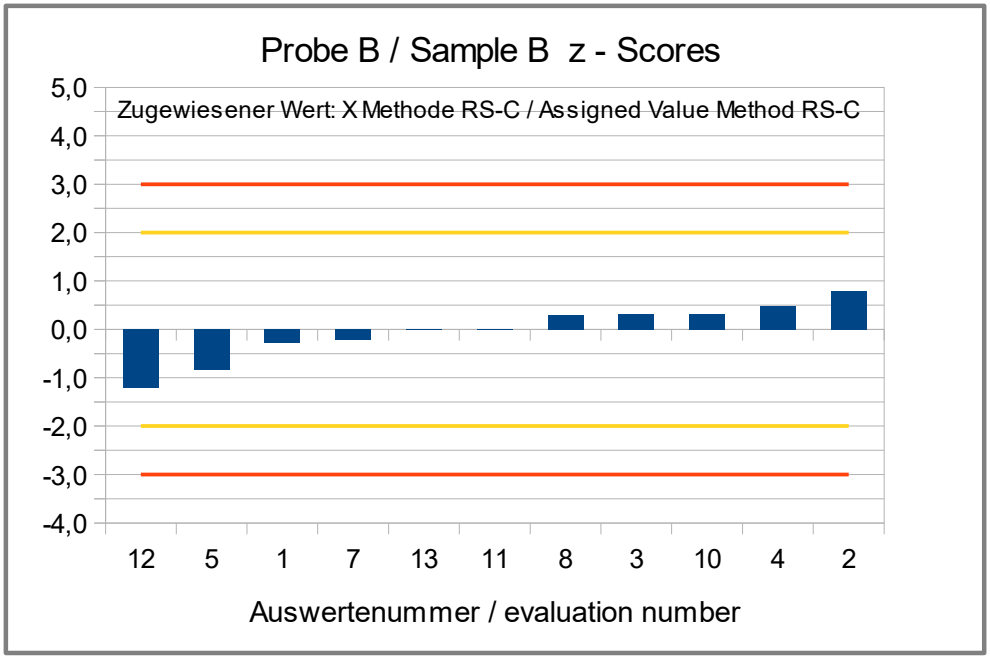


Abb./Fig. 4:
z-Scores ELISA Results Gluten, Assigned value robust mean of results method RS-C (R-Biopharm, Ridascreen competitive)

4.1.2 PCR Results: Gluten

No PCR results were submitted by the participants.

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: Gluten

Meth. Abbr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food/ protein	ELISA Test-Kit + Manufacturer
BF	15	12.06.20	negative	0	positive	108	0,36	2		Gluten	MonoTrace Gluten ELISA kit, BioFront Technologies
IL	6	30.04.	positive	10	positive	64	0,03*	0,2*		Gluten	Immunolab Gliadin/Gluten ELISA
RS-C	1	15.04.20, 30.04.20	negative	<10	positive	60	10	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	2	14.4./15.4.	-	10	-	77,30	4,6	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	3		negative	< 10	positive	69,53		10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	4	04.05.20	negative	<5	positive	72,1	5	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	5	15.05.20	negative	<10	positive	51	10	10	46	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	7	24.04.20	negative		positive	60,8	10	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	8	25.05.20	positive	19,7	positive	69,2	8	10	50	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	10	24.04.20	negative	< 10	positive	69,54	4,6	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	11	5. May	negative	<10	positive	64,7	10	10	38,11	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	12	24.04.20	negative		positive	44,73	10	10	18	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	13	02.06.20	negative		positive	64,56	10	10	59,13	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	14	10.06.20	negative	<10	positive	123,1	4,6	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
SP	9	16.04.20	negative	<5	positive	21	5	5		Gluten	SENSISPEC Ingezim Test-Combination 30.GLH.K2:2015
VT-R5	16	14.05.20	-	0,8	-	15,9				Gluten	Veratox Gliadin R5, Neogen

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

Continuation ELISA Gluten:

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	
BF	15	Anti-Gliadin Monoclonal	1:40 extraction ratio for 1 hour @ 60c	no	
IL	6	polyklonal	Initial result was converted by factor 20 due to 5% reactivity of barley compared to wheat according Instruction for Use of the ELISA.		*only for beer, 0.3/2 ppm for other samples
RS-C	1		according to protocol for beer		
RS-C	2	monoclonal R5	according to test instructions	yes	
RS-C	3			yes	
RS-C	4	monoclonal R5 antibodies	Extraction with 60% ethanol solution,which contains 10% fish gelatin, according to the test instructions	yes	
RS-C	5	Antibody R5 -Prolamins (QQPFP)	Extraction: 1 mL in 60%Etanol/10%fish gelatin. 10 minutes agitation-10 minutes centrifugation. Final Dilution 1:500 in sample buffer	No	Results expresed as gluten (2Xgliadin)
RS-C	7	R5-Antibody (detected gliadin, secalin, hordein)		no	
RS-C	8	R5	1ml sample (in duplicates) +9ml 60%EtOH with 10%fishgelatin. Vortex well, shake at RT for 10 min. Centrifuge 2500g 10min. The supernatant is diluted 1:50 before ELISA (20uL+980uL dilution buffer).	yes	kitlot used: 12429
RS-C	10	monoclonal R5	Extraction and determination were carried out according to the manufacturer's instructions	yes	
RS-C	11	Prolamin	Gluten equivalence reported from detected gliadin	yes	
RS-C	12	monoclonal R5	ethanol solution (60%) containing 10% liquid fish gelatin	yes	
RS-C	13			yes	
RS-C	14	R5	60% ethanol solution with 10% fish gelatin, 10min at RT shake upside down	no	
SP	9	monoclonal, R5	according to manufacturer's instructions	yes	
VT-R5	16		15 min / 60°C		

5.2 Information on the Proficiency Test (PT)

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

PT number	***ptAL10 (2020)***
PT name	***Allergens X: Gluten in „gluten-free“ Beer***
Sample matrix (processing)	*Samples A + B:* *Ingredients: Water, barley malt, hops*
Number of samples and sample amount	*2 different Samples A + B: 50 ml each*
Storage	*Please cool samples on arrival (2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Gluten* *Samples A + B: < 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.* *Before analysis we recommend to shake the samples gently for homogenization.*
Result sheet	*One result each should be determined for Samples A and B.* *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 June 12th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		USA
		ITALY
		SWITZERLAND
		ITALY
		SWITZERLAND
		SPAIN
		Germany
		Germany
		Germany
		Germany
		Germany
		SWEDEN
		GREAT BRITAIN
		GREAT BRITAIN
		Germany
		Germany

[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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