Proficiency Tests
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Evaluation Report
proficiency test

DLA 10/2018

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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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 31 August 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

The test material was a common in commerce "gluten-free" beer (rice beer). The basic composition of both sample A and sample B was the same (see table 1).
After homogenization of the basic mixture sample A was spiked with a gluten-containing spiking sample (mixture of "gluten-free" beer and wheat beer) and homogenized again.

The samples were portioned to approximately 50 mL in PE bottles with screw lock.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**
Organic Rice Beer, gluten-free Labelling: 4,3%vol alcohol, draught beer Ingredients: Water, rice syrup, hops Preservative: potassium sorbate*	90 g/100 g	100 g/100 g
Gluten-containing Spiking Sample Ingredients: Mixture of "gluten free" Pilsner Beer (Lager) and Bright Wheat Beer (Hefeweißbier) with Wheat and Barley malt (DLA 10-2016 Sample A) - thereof Gluten**	10 g/100g 25 mg/kg	-

* preservation of PT-samples by DLA
** Gluten content according to final report of DLA 10-2016 sample A: robust mean 251 mg/kg, standard uncertainty 17,2 mg/kg (method ELISA: R-Biopharm, Ridascreen Gliadin competitive R7021)

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** (spiked sample A) 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].

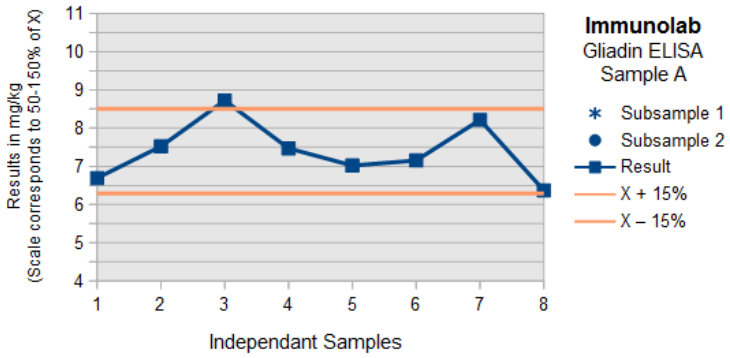
ELISA-Tests: Homogenität Gluten / Homogeneity Gluten

Immunolab Gliadin/Gluten ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Gluten 7,4 ± 0,77 mg/kg

Sample A	Subsample 1 mg/kg	Subsample 2 mg/kg	Result mg/kg
1	-	-	6,69
2	-	-	7,52
3	-	-	8,72
4	-	-	7,47
5	-	-	7,02
6	-	-	7,15
7	-	-	8,21
8	-	-	6,37

General average X	7,40	
SD of samples	0,773	10,5%



2.1.2 Stability

The food matrix sample material is beer. In long-term stability tests over one year, the parameter gluten has proved 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 20th week of 2018. The testing method was optional. The tests should be finished at June 29th 2018.

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

There are two different samples A and B with possible levels of gluten from barley and/or wheat malt (from Pilsner beer or yeast wheat beer) in the mg/kg range in the matrix "gluten-free" beer.

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 sent by email.
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 17 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 material sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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

3.1 Consensus value from participants (assigned value)

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

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

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

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

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

3.2 Robust standard deviation

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

The following robust standard deviations were considered:

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

3.3 Exclusion of results and outliers

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

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

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

3.4 Target standard deviation (for proficiency assessment)

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

3.4.1 General model (Horwitz)

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

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

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

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

3.4.2 Value by precision experiment

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

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

The 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 process, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision, and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [3].

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

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (xi) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

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

If carried out an evaluation of the results by means of z 'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and 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

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 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.

All gluten ELISA results were submitted 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 (X_{pt})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{METHOD\ i}}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* σ_{pt}'		
lower limit of target range (X_{pt} - $2\sigma_{pt}$) or (X_{pt} - $2\sigma_{pt}'$)°		
upper limit of target range (X_{pt} + $2\sigma_{pt}'$) or (X_{pt} + $2\sigma_{pt}'$)°		
Quotient S*/σ_{pt} *or* S*/σ_{pt}'		
Standard uncertainty $U_{(Xpt)}$		
Number of results in target range		
Percent in target range		

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

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		
17	positive	10,0	positive	4,00	1/2 (50%)	IL	
7a	positive	11,0	negative	<5	2/2 (100%)	IN	
10a	positive		negative		2/2 (100%)	RQ	Lateral Flow
1	positive	31,0	negative		2/2 (100%)	RS-C	
3	positive	13,0	negative	<10	2/2 (100%)	RS-C	
4	positive	12,6	negative	<10	2/2 (100%)	RS-C	
5	positive	41,0	negative	<10	2/2 (100%)	RS-C	
6	positive	20,6	negative	<10,0	2/2 (100%)	RS-C	
7b	positive	21,0	negative	<10	2/2 (100%)	RS-C	
8	positive	20,4	negative		2/2 (100%)	RS-C	
9	positive	19,0	negative		2/2 (100%)	RS-C	
10b	positive	52,0	negative	<5	2/2 (100%)	RS-C	
11	positive	20,1	negative	<10	2/2 (100%)	RS-C	
12	positive	12,4	negative	<10	2/2 (100%)	RS-C	
13	positive	10,7	negative	<10,0	2/2 (100%)	RS-C	
14	positive	10,2	negative	<10.0	2/2 (100%)	RS-C	
15	positive	12,0	negative		2/2 (100%)	RS-C	
16	positive	22,0	negative		2/2 (100%)	RS-C	
2	positive	12,1	negative	<5,0	2/2 (100%)	VT-R5	

	Sample A		Sample B	
Number positive	19		1	
Number negative	0		18	
Percent positive	100		5	
Percent negative	0		95	
Consensus value	positive		negative	

Methods:

IL = Immunolab
IN = INgezim Gluten Hidrolizado, Ingenasa
RQ = RIDA®QUICK (Lateral Flow), R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
VT-R5 = Veratox, Neogen

Comments:

The consensus values are in agreement with the spiking of sample A. One positive result for sample B was obtained by method IL at the LOQ of the method. The result was below the LOQs indicated by the participants for the other methods.

Quantitative valuation of ELISA-results: Sample A

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}C}$	Method	Remarks
	[mg/kg]				
17	10,0	-1,5		IL	
7a	11,0	-1,2		IN	
10a				RQ	Lateral Flow
1	31,0	3,9	3,3	RS-C	
3	13,0	-0,69	-0,92	RS-C	
4	12,6	-0,79	-1,0	RS-C	
5	41,0	6,4	5,7	RS-C	outlier excluded
6	20,6	1,2	0,87	RS-C	
7b	21,0	1,3	1,0	RS-C	
8	20,4	1,2	0,83	RS-C	
9	19,0	0,83	0,50	RS-C	
10b	52,0	9,2	8,3	RS-C	outlier excluded
11	20,1	1,1	0,76	RS-C	
12	12,4	-0,83	-1,1	RS-C	
13	10,7	-1,3	-1,5	RS-C	
14	10,2	-1,4	-1,6	RS-C	
15	12,0	-0,95	-1,2	RS-C	
16	22,0	1,6	1,2	RS-C	
2	12,1	-0,92		VT-R5	

Method:
IL = Immunolab
IN = INgezim Gluten Hidrolizado, Ingenasa
RQ = RIDA®QUICK (Lateral Flow), R-Biopharm
RS-C = Ridascreen® competitive, R-Biopharm
VT-R5 = Veratox, Neogen

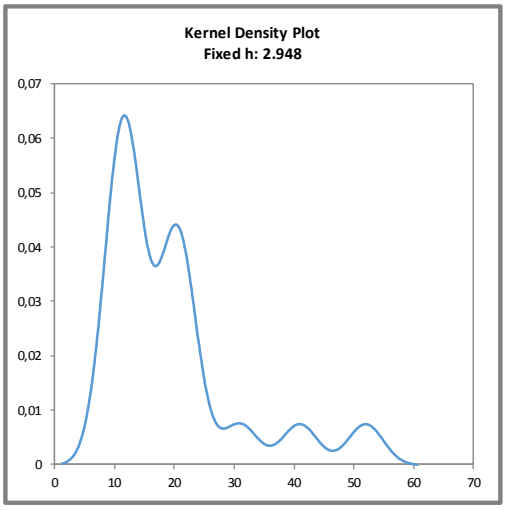


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows a distribution with two maxima of results and three minor peaks, due to single results above the target range.

Characteristics: Quantitative evaluation ELISA Gluten

Sample A

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	13
Number of outliers	-	-
Mean	16,1	17,3
Median	12,8	19,0
Robust Mean (X_{pt})	**15,7**	**16,9**
Robust standard deviation (S*)	**5,72**	**5,87**
Target range:		
Target standard deviation σ_{pt}	**3,93**	**4,23**
lower limit of target range	**7,86**	**8,45**
upper limit of target range	**23,6**	**25,4**
Quotient S/σ_{pt}*	*1,5*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*1,79*	*2,04*
Results in the target range	*15*	*12*
Percent in the target range	*94*	*92*

* without results 5 and 10b (excluded in advance)

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences. The observed second maximum was due to about half of the results of method RS-C. The results of method RS-C gave for the first peak a mean of 11,8 ± 1,3 mg/kg (n=6) and for the second peak 20,5 ± 1,1 mg/kg (n=6). From the information provided by the participants (including extraction solutions, date of analysis, location of the laboratory), no causes can be derived (see documentation). Remarkable is only the factor of about 2, which corresponds to the conversion of gliadin to gluten. However, all participants with one exception reported the results as gluten in the result submission file. Since the resulting statistical characteristics were inconspicuous, nevertheless, a joint evaluation of results was made. Two outliers were excluded in advance.

The evaluation of results of all methods and method RS-C showed a normal variability of results. The quotients S/σ_{pt} were below 2,0. The robust standard deviations are 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. The special features of distribution of the RS-C results mentioned above should be noted.*

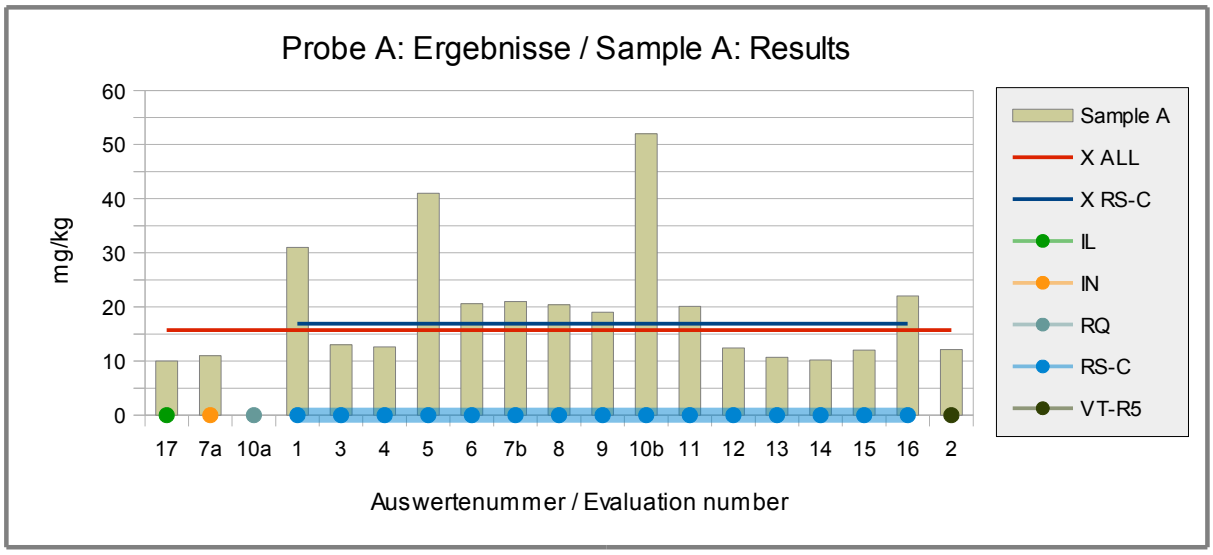


Abb./Fig. 2: ELISA Results Gluten
red line = Assigned value robust mean all results
blue line = Assigned value robust mean method RS-C
round symbols = Applied methods (see legend)

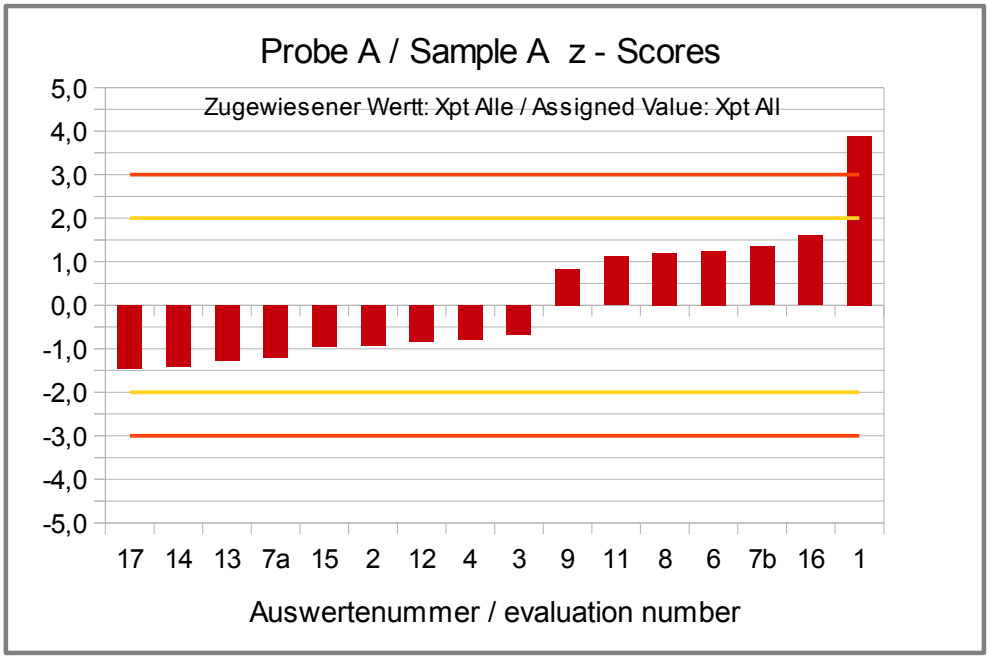


Abb./Fig. 3:
z-Scores (ELISA Results Gluten) Assigned value robust mean of all results

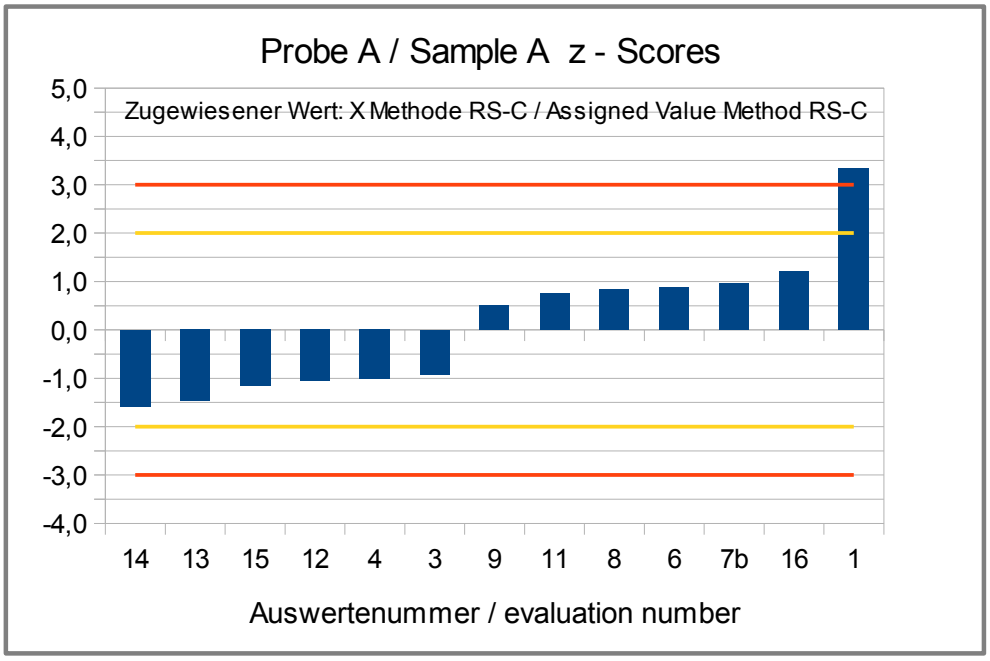


Abb./Fig. 4:
z-Scores (ELISA Results Gluten) Assigned value robust mean of results method RS-C (R-Biopharm, Ridascreen competitive)

4.1.2 PCR Results: Wheat DNA

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		
10	-		-			div	

Methods:
div = not indicated / other method

Comments:
One participant tried to analyse the samples A and B by a PCR method. No amplifiable wheat DNA was obtained.

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. Abr.	Evaluation number	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	Test-Kit + Manufacturer
IL	17	22.05.18	positive	10	positive	4	0,06	4		Gluten	Immunolab Gliadin/Gluten ELISA
IN	7a	27.6.	positive	11	negative	<5	5	5		Gluten	Ingezim Gluten Hidrolizado R.30.HLH.K2/48
RQ	10a	12.06.18	positive		negative		6,3			Gluten	RIDA QUICK Gliadin R7003, R-Biopharm
RS-C	1	11.06.18	positive	31	negative			10		mg Gluten/kg Food	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	3	29.05.18	positive	13	negative	< 10		< 10	50	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	4	18.05.18	positive	12,61	negative	<10	10	10	12,64	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	5	26.06.18	positive	41	negative	< 10				Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	6	21.05.18	positive	20,6	negative	<10,0					R-Biopharm, R7021
RS-C	7b	4.6.	positive	21	negative	<10	10	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	8	20.06.18	positive	20,4	negative		10	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	9	20.06.18	positive	19	negative			10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	10b	12.06.18	-	52	-	< 5	5	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	11	22.5.18/ 07.06.18	-	20,1	-	< 10	4,6	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	12	31.05.18	positive	12,44	negative	<10		10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	13	31/05	positive	10,7	negative	<10,0	10	10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	14	06.06.18	positive	10,2	negative	<10.0		10		Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	15	17.05.18	positive	12	negative		10	10	35	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
RS-C	16	17.05.18	positive	22	negative		5	10	25	Gluten	Ridascreen® Gliadin competitive R7021, R-Biopharm
VT-R5	2	28.06.18	positive	12,1	negative	<5,0	2	3,73	12,9	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. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
IL	17	polyclonal			Sampe B slightly above LOQ
IN	7a		As per kit instructions	yes	
RQ	10a	R5	Extraction with 60% Ethanol Solution containing 10 % Fish Gelatin, according to test kit instructions	yes	
RS-C	1	R5	Ethanol		
RS-C	3		according to manual, with fish gelatin	yes	
RS-C	4	As per kit instructions	As per kit instructions	No	
RS-C	5	R5	60% ethanol + fish gelatin / 20 min / room temperature	yes	LAB result
RS-C	6			yes	
RS-C	7b	monoclonal R5	As per kit instructions	yes	
RS-C	8			yes	
RS-C	9			no	
RS-C	10b	R5	Extraction with 60% Ethanol Solution containing 10 % Fish Gelatin, according to test kit instructions	yes	
RS-C	11	monoclonal R5	As per kit instructions	yes	
RS-C	12			yes	
RS-C	13			NO	
RS-C	14				
RS-C	15	monoclonal antibody R5	according to handbook	yes	
RS-C	16	R5	ETOH 60% +Gel fish /10min/22°C		
VT-R5	2	R5	0,25 g + 2,5 mL renaturing cocktail solution (w / extraction additive); incubation at 50°C, 40 min; final volume 10 mL; dilution 1:12,5.	NO	Meas & calc with ELISA Reader Neogen Stat Fax 4700

5.1.2 PCR: Wheat DNA

Meth. Abr.	Evaluation number	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 / food protein	Test-Kit + Manufacturer
div	10	25.05.18	-		-					wheat DNA	Alary et al. 2002, Cereal Chem. 79: 553-558

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
div	10	Lipid Transfer Protein Gene (ltp)	CTAB Precipitaion, QIAgen PCR Purification Kit	no	with the applied method no amplificable DNA was isolated, it could not be assessed wether wheat is present or not

5.2 Information on the Proficiency Test (PT)

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

PT number	***DLA 10-2018***
PT name	***Allergens X: Gluten in „gluten-free“ Beer***
Sample matrix (processing)	*Samples A + B:* *Ingredients: Water, rice syrup, hops and one sample A or B additionally barley malt and wheat malt*
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 Sample A and Sample 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***
Deadline	***the latest June 29th 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		ITALY
		Germany
		Germany
		Germany
		Germany
		CANADA
		Germany
		Germany
		Germany
		Germany
		ARGENTINIA
		GREAT BRITAIN
		Germany
		FRANCE
		SPAIN
		CANADA
		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

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
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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
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