DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

10/2016

Allergens X:

Gluten in "gluten-free" Beer

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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 are common in commerce german beers and mixtures of them, respectively. The varieties "gluten-free" beer, Pilsner beer and wheat beer were used for mixing the test samples as indicated in Table 1. For preservation of samples potassium sorbate was added.

After homogenisation 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**	**Sample C**
"Gluten-free" Pilsner Beer (Lager) Labelling: 4,7%vol alcohol, 11,5% original wort Ingredients: mineral water, **barley malt**, hops Preservative: potassium sorbate *	81 g/100g	100 g/100g	50 g/100g
Pilsner Beer (Lager) Labelling: 4,7%vol alcohol, 11,5% original wort Ingredients: mineral water, **barley malt**, hops Preservative: potassium sorbate *	-	-	50 g/100g
Bright Wheat Beer (Helles Hefe-weißbier) Labelling: 5,1%vol alcohol, 12,5% original wort Ingredients: mineral water, **wheat malt**, **barley malt**, hops, yeast Preservative: potassium sorbate *	19 g/100g	-	-

* preservation of PT-samples by DLA

2.1.1 Homogeneity

Homogeneity of sample A was checked by ELISA-test for gluten (fig. 1). The resulting standard deviation between the samples of < 15% ensured sufficient homogeneity [14, 15, 18, 19]. 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 (s. 3.8 and 3.11) [3].

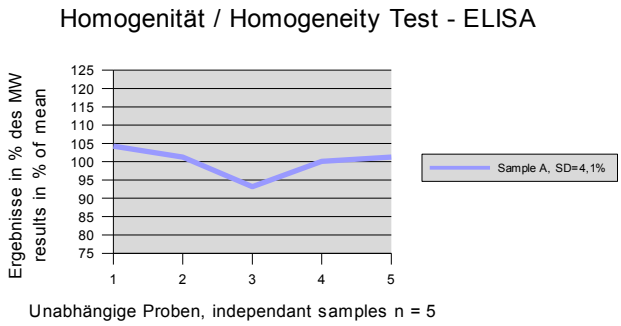

Fig. 1: Testing of homogeneity of DLA-sample A
Results are given in percent of the arithmetic mean

2.2 Sample shipment and information to the test

The portions of test material (samples A, B and C) were sent to every participating laboratory in the 20th week of 2016. The testing method was optional. The tests should be finished at July 1st 2016 the latest.

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

Important Note: Please cool samples on arrival (2 - 10 °C). Before analysis we recommend to shake the samples samples gently for homogenization.

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredient gluten in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specifity, 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.

20 participants submitted their results in time. Two participants 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 [21, 22, 23, 24, 26]. 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.

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].
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) ***Robust mean of all results*** - $X_{pt_{ALL}}$
ii) ***Robust mean 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]. 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 identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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-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]:

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

Because in the present proficiency test the number of replicate measurements is n = 1, the reproducibility standard deviation σ_R is identical to the target standard deviation σ_{pt}.

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 [20]. 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 [20].

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) [25]. "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 [25]

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

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) [26].

Colgrave et al. (2014) applied a LC-MS/MS method for the determination of gluten present in hydrolyzed form in beer in comparison to ELISA methods [27].

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 [18], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [15-17], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [19] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [14].

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 [14-20]	**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 [14]	**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 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. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and 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 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].

3.7 Standard uncertainty of the assigned value

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 Quotient $U_{(Xpt)}/\sigma_{pt}$ is reported in the characteristics of the test.

3.8 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.
The following result sections are structured equally for the allergenic components. First all results for a certain analyte are reported together for sample A and afterwards for sample B.

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, which were given as gluten, were converted into gliadin using a factor of 2 (gliadin x 2 = gluten).

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.

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]	X All	X Method i		

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		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data:		
Target standard deviation σ_{pt}		
lower limit of target range (X_{pt} - 2σ_{pt})		
upper limit of target range (X_{pt} + 2σ_{pt})		
Quotient S*/σ_{pt}		
Standard uncertainty $U_{(Xpt)}$		
Quotient $U_{(Xpt)}/\sigma_{pt}$		
Number of results in target range		
Percent in target range		

4.1 Proficiency Test Gluten

4.1.1 ELISA-Results: Gluten

Qualitative valuation of results: Samples A, B and C

Evaluation number	Sample A	Sample A	Sample B	Sample B	Sample C	Sample C	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
1	positive	534,1	negative	< 1	positive	1,75	3/3 (100%)	BC	outlier X_{All}
14	positive	329	positive	5,4	positive	16	3/3 (100%)	IG	
20	positive	186	positive	2	positive	2,6	3/3 (100%)	IL	Result converted *
2	positive	330	negative	< 10	positive	17	3/3 (100%)	RS1	
3	positive	258	positive	10	positive	19	3/3 (100%)	RS1	
4	positive	202	negative	< 10	positive	10,14	3/3 (100%)	RS1	
5	positive	> 270	negative	< 10	positive	17,3	3/3 (100%)	RS1	Result converted *
6	positive	198,3	negative		positive	14,5	3/3 (100%)	RS1	
7	positive	193,87	negative	< 10	positive	18,98	3/3 (100%)	RS1	
8	positive	250	negative	< 2,72	positive	34	3/3 (100%)	RS1	
9	positive	223,74	negative		positive	13,31	3/3 (100%)	RS1	
10	positive	279,50	negative	< 10,0	positive	25,54	3/3 (100%)	RS1	Result converted *
11	positive	242,7	negative	< 10	positive	18,3	3/3 (100%)	RS1	mean calculated by DLA
12	positive	218	negative	< 2,72	positive	16	3/3 (100%)	RS1	Result converted *
13	positive	295	negative	< 10	positive	23	3/3 (100%)	RS1	
15	positive	268,95	negative	< 3	positive	26,29	3/3 (100%)	RS1	
16	positive	282,75	positive	18,16	positive	32,21	3/3 (100%)	RS1	
17	positive	471	positive	17	positive	30	3/3 (100%)	RS1	outlier X_{All} a. X_{RS1}
18	positive	188	negative	< 10	positive	13	3/3 (100%)	RS1	
19	positive	81	negative	< 5	negative	< 5	2/3 (67%)	RS2	

* calculation see p. 13

	Sample A		Sample B		Sample C	
Number positive	20		5		19	
Number negative	0		15		1	
Percent positive	100		25		95	
Percent negative	0		75		5	
Consensus value	positive		corresponds	< 20	positive	

Methods:

BC = Bio-Check, Tecna
IG = Ingenasa
IL = Immunolab
RS1 = Ridascreen Gliadin competitive R7021, R-Biopharm
RS2 = Ridascreen Gluten R7001, R-Biopharm

Comments:

There were 100% positive results for the detection of gluten by ELISA methods in samples A and C. The consensus values are in qualitative agreement with the ingredients of the samples (Pilsner beer and wheat beer).

For sample B there were 75% negative and 25% positive results. All results were below 20 mg/kg in agreement with the labeling as "gluten-free". For qualitative valuation of the results for sample B the agreement with a content <20 mg/kg was used as consensus.

Quantitative valuation of results: Sample A

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS1}	Method	Remarks
	[mg/kg]				
1	534,1	4,4		BC	outlier X_{All}
14	329	1,2		IG	
20	186	-1,1		IL	Result converted *
2	330	1,2	1,3	RS1	
3	258	0,1	0,1	RS1	
4	202	-0,8	-0,8	RS1	
5	> 270			RS1	Result converted *
6	198,3	-0,9	-0,8	RS1	
7	193,87	-0,9	-0,9	RS1	
8	250	0,0	0,0	RS1	
9	223,74	-0,5	-0,4	RS1	
10	279,50	0,4	0,5	RS1	Result converted *
11	242,7	-0,2	-0,1	RS1	mean calculated by DLA
12	218	-0,6	-0,5	RS1	Result converted *
13	295	0,7	0,7	RS1	
15	268,95	0,2	0,3	RS1	
16	282,75	0,5	0,5	RS1	
17	471	3,4	3,5	RS1	outlier X_{all} a. X_{RS1}
18	188	-1,0	-1,0	RS1	
19	81	-2,7		RS2	

* calculation see p. 13

Methods:

BC = Bio-Check, Tecna
IG = Ingenasa
IL = Immunolab

RS1 = Ridascreen Gliadin competitive R7021, R-Biopharm
RS2 = Ridascreen Gluten R7001, R-Biopharm

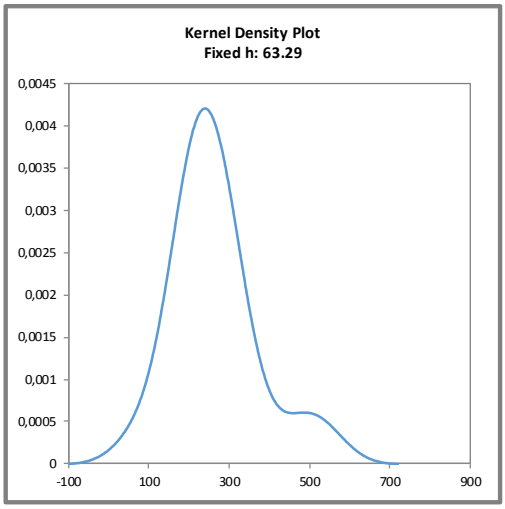

Fig. 2: Kernel Density Plot of all ELI-SA-results gluten
(mit h = σpt von Xpt_{ALL})

Comments:
The kernel density estimation shows a normal distribution with an additional minor peak at 500 mg/kg due to the two outliers (s. fig. 2).

Characteristics: Quantitative evaluation Gluten

Sample A

Characteristics	**All Results** *[mg/kg]*	**Method RS1** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS1}}$
Number of results	*19*	*15*
Number of outliers	*2*	*1*
Median	250	250
Robust mean (X_{pt})	253	251
Robust standard deviation (S*)	69,9	53,4
Target data:		
Target standard deviation σ_{pt}	63,3	62,7
lower limit of target range (X_{pt} - 2σ_{pt})	127	125
upper limit of target range (X_{pt} + 2σ_{pt})	380	376
Quotient S/σ_{pt}*	*1,1*	*0,85*
Standard uncertainty $U_{(Xpt)}$	*20,0*	*17,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,32*	*0,28*
Number of results in target range	*16*	*14*
Percent in target range	*84%*	*93%*

Method:
RS1 = R-Biopharm, Ridascreen Gliadin competitive R7021

Comments to the statistical characteristics:

The evaluation of results of all methods and of method RS1 showed a low variability. The quotients S/σ_{pt} were clearly below 2,0. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied method (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given.*

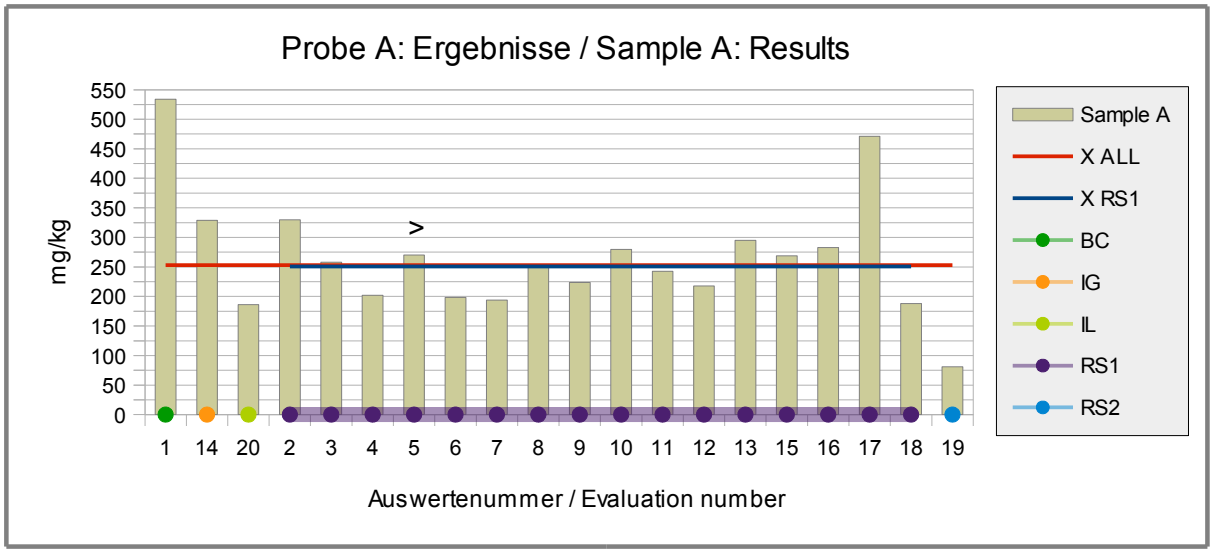

Fig. 3: ELISA-Results Gluten
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS1
round symbols = Applied methods (see legend)

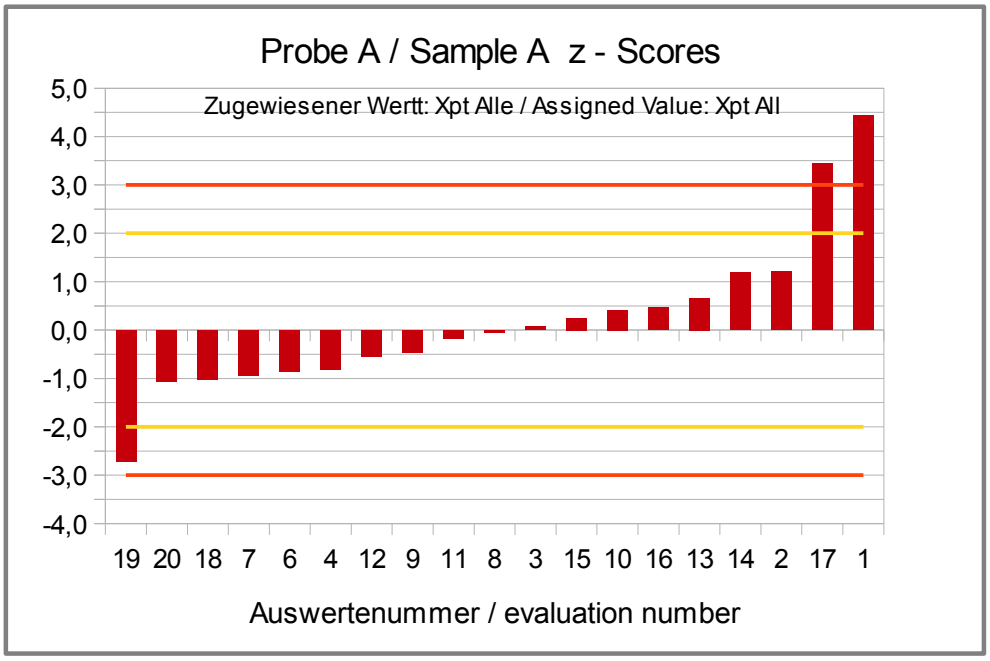

Fig. 4: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of all results

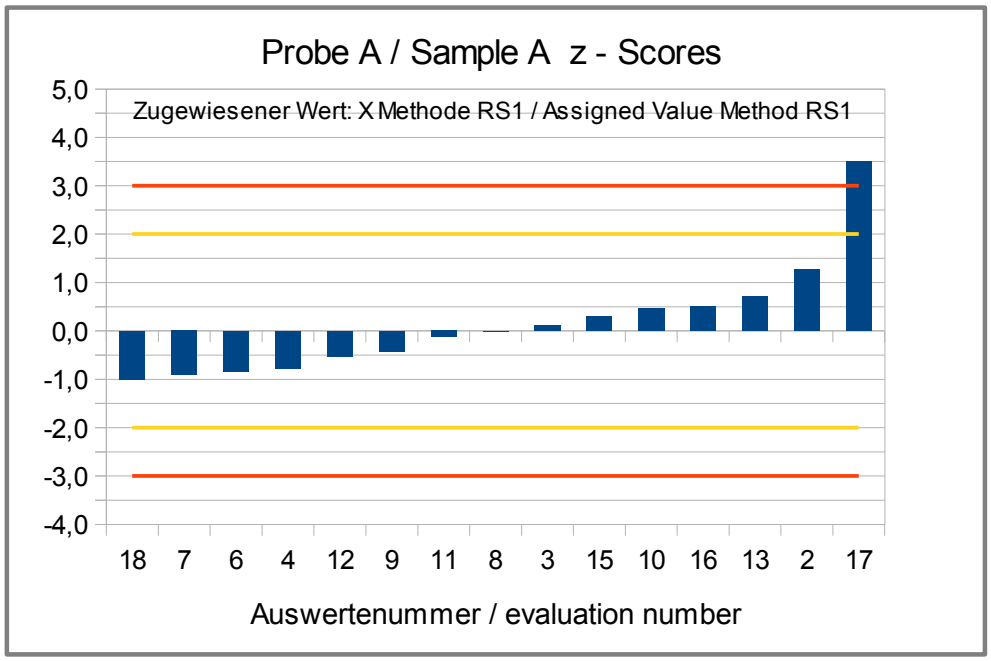

Fig. 5: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of method RS1
(R-Biopharm, Ridascreen competitive)

Quantitative valuation of results: Sample C

Evaluation number	Gluten	z-Score Xpt_{ALL}	z-Score Xpt_{RS1}	Method	Remarks
	[mg/kg]				
1	1,75	-3,6		BC	
14	16	-0,6		IG	
20	2,6	-3,4		IL	Result converted *
2	17	-0,3	-0,7	RS1	
3	19	0,1	-0,3	RS1	
4	10,14	-1,8	-2,0	RS1	
5	17,3	-0,3	-0,6	RS1	Result converted *
6	14,5	-0,9	-1,2	RS1	
7	18,98	0,1	-0,3	RS1	
8	34	3,3	2,7	RS1	
9	13,31	-1,1	-1,4	RS1	
10	25,54	1,5	1,0	RS1	Result converted *
11	18,3	-0,1	-0,4	RS1	mean calculated by DLA
12	16	-0,6	-0,9	RS1	Result converted *
13	23	1,0	0,5	RS1	
15	26,29	1,7	1,1	RS1	
16	32,21	2,9	2,3	RS1	
17	30	2,5	1,9	RS1	
18	13	-1,2	-1,5	RS1	
19	< 5			RS2	

* calculation see p. 13

Methods:

BC = Bio-Check, Tecna
IG = Ingenasa
IL = Immunolab

RS1 = Ridascreen Gliadin competitive R7021, R-Biopharm
RS2 = Ridascreen Gluten R7001, R-Biopharm

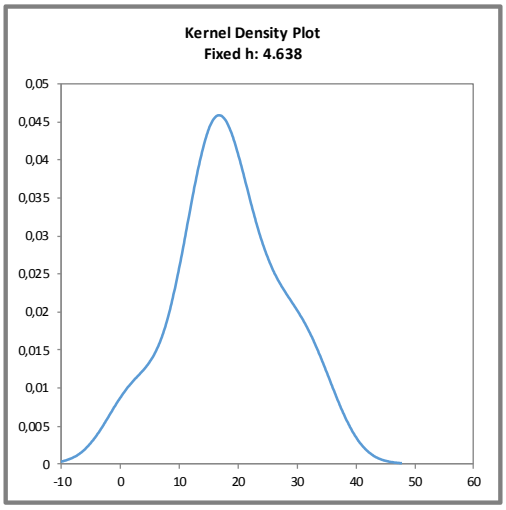

Fig. 6: Kernel Density Plot of all ELI-SA-results gluten
(mit h = σpt von Xpt_{ALL})

Comments:
The kernel density estimation shows a normal distribution with two shoulders at appr. 2 mg/kg (methods BC and IL) and 30 mg/kg (s. fig. 6).

Characteristics: Quantitative evaluation Gluten

Sample C

Characteristics	**All Results** *[mg/kg]*	**Method RS1** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS1}}$
Number of results	*19*	*16*
Number of outliers	*0*	*0*
Median	17,3	18,6
Robust mean (X_{pt})	18,6	20,4
Robust standard deviation (S*)	9,17	7,95
Target data:		
Target standard deviation σ_{pt}	4,64	5,11
lower limit of target range ($X_{pt} - 2\sigma_{pt}$)	9,28	10,2
upper limit of target range ($X_{pt} + 2\sigma_{pt}$)	27,8	30,6
Quotient S/σ_{pt}*	*2,0*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*2,63*	*2,48*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,57*	*0,49*
Number of results in target range	*14*	*14*
Percent in target range	*74%*	*88%*

Method:
RS1 = R-Biopharm, Ridascreen Gliadin competitive R7021

Comments to the statistical characteristics:

The evaluation of results of all methods and of method RS1 showed a normal variability. The quotients S/σ_{pt} were at 2,0 and below 2,0, respectively. The robust standard deviation is in the range of established values for the reproducibility standard deviation of the applied method (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given.*

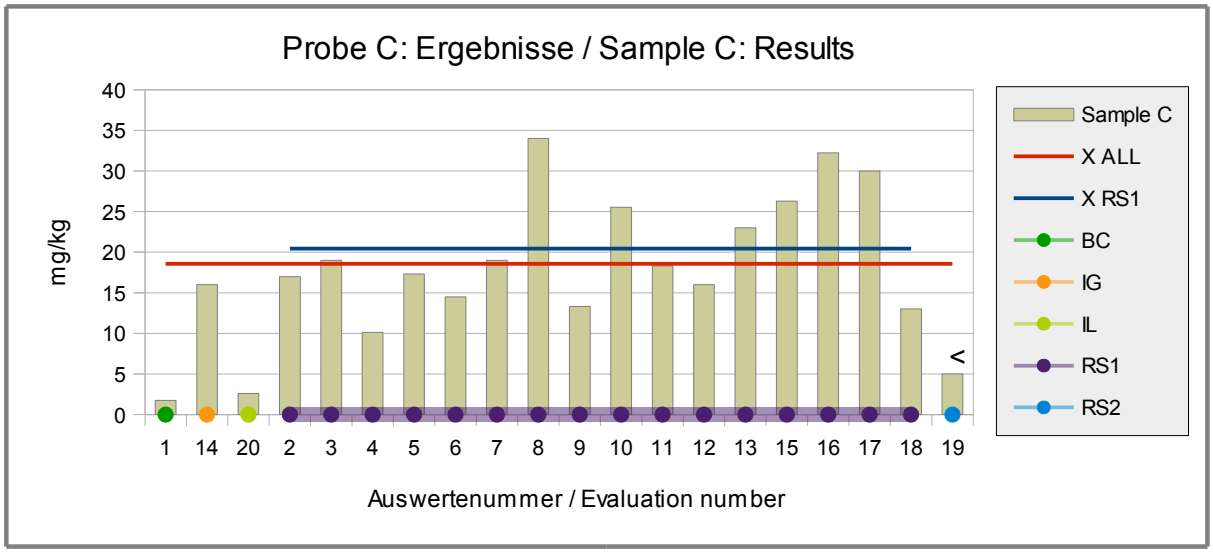

Fig. 7: ELISA-Results Gluten
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS1
round symbols = Applied methods (see legend)

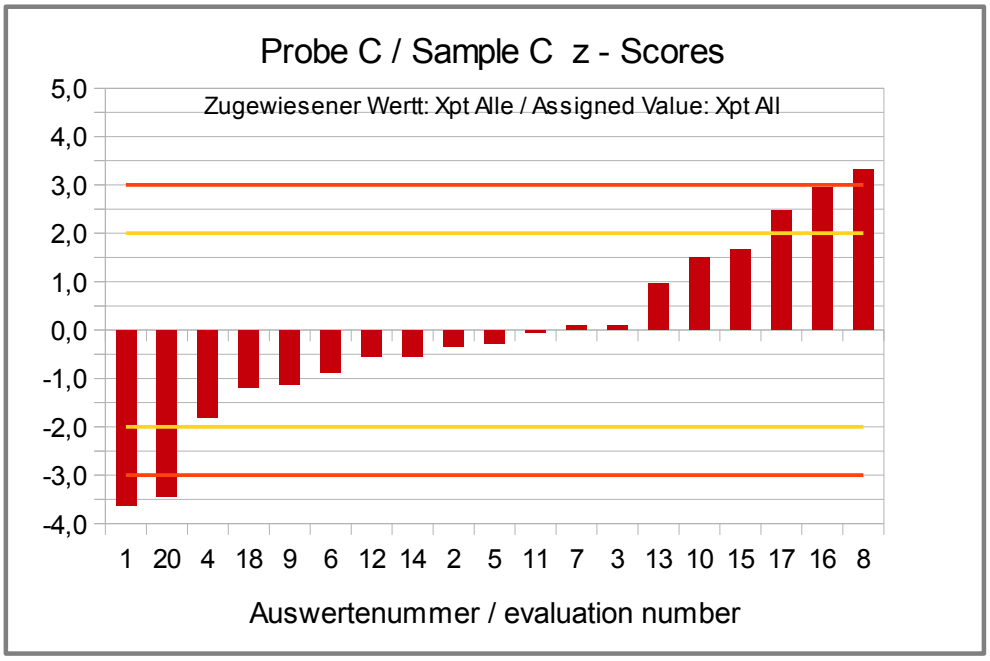

Fig. 8: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of all results

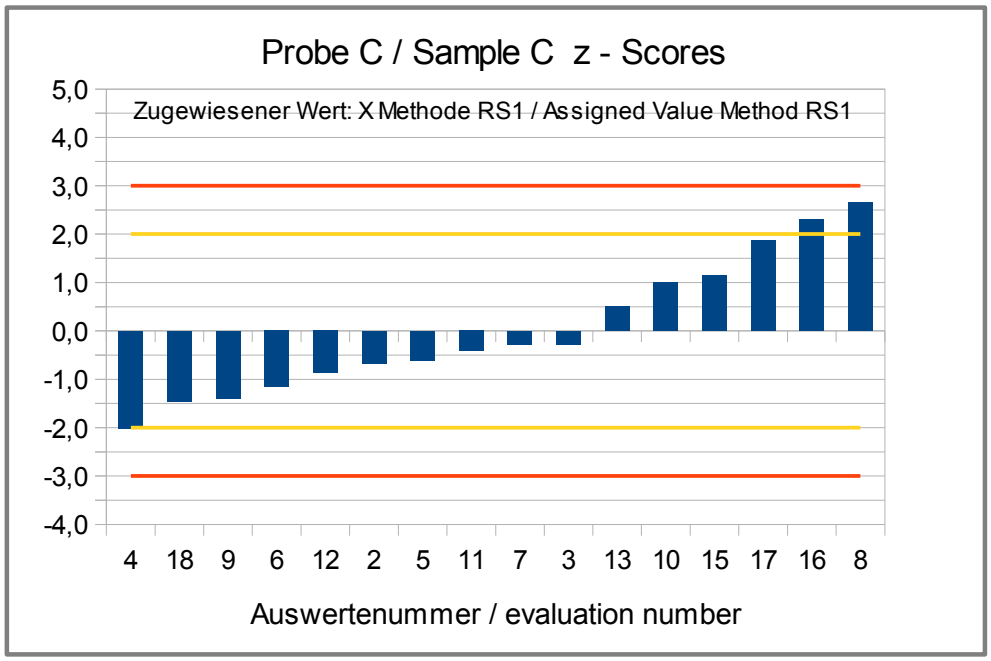

Fig. 9: z-Scores (ELISA-Results as Gluten)
Assigned value robust mean of method RS1
(R-Biopharm, Ridascreen competitive)

4.1.2 PCR-Results: Gluten-containing Cereals

Qualitative valuation of results: Samples A, B and C

Evaluation number	Sample A	Sample A	Sample B	Sample B	Sample C	Sample C	Qualitative Valuation	Method	Remarks
	pos / neg	mg/kg	pos / neg	mg/kg	pos/neg	[mg/kg]	Agreement with Consensus Value		
8	negative	< 0,4	negative	< 0,4	negative	< 0,4	-	SFA-ID	
9	negative		negative		negative		-	div	

Methods:

SFA = Sure Food Allergen, R-Biopharm / Congen

div = not indicated / other method

Comments:

There were exclusively negative results for the detection of DNA from gluten-containing cereals by PCR methods.

5. Documentation

Details by the participants

5.1 ELISA: Gluten

Primary data

Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Sample C		quantitative Result given as	Meth. Abr.
	day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	e.g. food / food protein	
1	01.07.16	positive	534,1	negative	< 1	positive	1,75	Gluten	BC
14	06.06.16	positive	329	positive	5,4	positive	16	Gluten	IG
20	20.05.16	positive	93	positive	1	positive	1,3	Wheat-Gliadin	IL
2	09.06.	positive	330	negative	<10	positive	17		RS1
3	29.06.16	positive	258		10	positive	19	Gluten	RS1
4	25.05.16	positive	202	negative	<10	positive	10,14	Gluten	RS1
5	30.06.	-	>135	-	<5	-	8,64	Gliadin	RS1
6	29.06.16	positive	198,3	negative		positive	14,5	Gluten	RS1
7	25.06.16	positive	193,87	negative	<10	positive	18,98	Gluten	RS1
8	13.06.16	positive	250	negative	< 2.72	positive	34	Gluten	RS1
9	22.06.16	positive	223,74	negative		positive	13,31	Gluten	RS1
10	08.06.16	positive	139,75		< 5,00	positive	12,77	Gliadin	RS1
11	28.06.16	positive	236,8	negative	< 10	positive	16,4	Gluten	RS1
11	28.06.16	positive	239,6	negative	< 10	positive	17,2	Gluten	RS1
11	28.06.16	positive	252,4	negative	< 10	positive	18	Gluten	RS1
11	28.06.16	positive	242	negative	< 10	positive	21,6	Gluten	RS1
12	27.06.16	-	109	-	< 1,36	-	8	Gliadin	RS1
13	9 june	-	295	-	<10	-	23	Gluten	RS1
15	10.06.	positive	268,95	negative	< 3	positive	26,29	Gluten	RS1
16	03.06.16	positive	282.75	positive	18.16	positive	32.21	Gluten	RS1
17	31.05.16	-	471	-	17	-	30	Gluten	RS1
18	25.05.16	positive	188	negative	<10	positive	13	Gluten	RS1
19	31.05.16	-	81	-	<5	-	<5		RS2

Methods:

BC = Bio-Check, Tecna
IG = Ingenasa
IL = Immunolab
RS1 = Ridascreen Gliadin competitive R7021, R-Biopharm
RS2 = Ridascreen Gluten R7001, R-Biopharm

Other details to the Methods

Evaluation number	Meth. Abr.	Method	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Test-Kit + Manufacturer	Antibody	e.g. Extraction Solution / Time / Temperature	
1	BC	BIO-CHECK (TECNA)	monoclonal antibodies	extraction solution / 45'/55°C	
14	IG	other: INGENASA	R5	Extraction with ETOH 60 %	
20	IL	Immunolab Gliadin GLU-E02	Gliadin	cross-reactivity to barley-gliadin: 5%	
2	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm			
3	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin	as per Kit Instructions	only sample A is to complain about; B at LOQ
4	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5	1ml sample + 9ml ethnolic fish gelatin buffer used	
5	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin	60% Ethanol + 10% Fish gelatin/10 min/RT	
6	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm		As per Kit Instructions	
7	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5 monoclonal antibody for potentially toxic peptide sequences of gliadins from wheat and prolamins from rye and barley	As per Kit Instructions	
8	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm			there is not a sample called Spiking Sample, but there is Sample C, I put the result for Sample C in result Spiking Sample
9	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin	as per Kit Instructions	
10	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin	as per Kit Instructions	
11	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5		Gluten - LAB
11	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5		Gluten -GE
11	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5		Gluten -VA
11	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5		Gluten -VL
12	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin		
13	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5 monoclonal antibody	1 ml sample + 9 ml ethanol solution (60%) containing 10% fish gelatin. Vortex for 30 sec before shaking on a rotator for 10 min. Centrifuge for 10 min in r.t at 2500 g. The supernatant is diluted 1:50 with diluted sample diluent before ELISA.	
15	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	Gliadin	as per test instruction for polyphenol containing solid samples: 60 % Ethanol, 10 % Fish gelatin	
16	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5	ethanol solution (60 %) containing 10 % fish gelatine/10 min/room temperature	Result Spiking Sample=Sample C
17	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R 5	as per Kit Instructions	
18	RS1	Ridascreen Gliadin competitive (R7021), r-Biopharm	R5		
19	RS2	Ridascreen Gluten (R7001), r-Biopharm	R5	cocktail solution used for extraction	

5.2 PCR: Gluten-containing Cereals

Primary data

Evaluation number	Date of Analysis	Result Sample A		Result Sample B		Result Sample C		quantitative Result given as	Meth. Abr.
	day/month	qualitative	mg/kg	qualitative	mg/kg	qualitativ	mg/kg	e.g. food / food protein	
8	13.06.16	negative	< 0.4	negative	< 0.4	negative	< 0.4	Wheat, rye, barley, oat, spelt, kamut DNA	SFA-ID
9		-		-		-			div

Methods:

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

Other Remarks to the Methods

Evaluation number	Meth. Abr.	Method	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Test-Kit + Manufacturer	Antibody	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
8	SFA-ID	Sure Food Allergen ID, Congen / r-Biopharm			
9	div		Lipidtransferase(Ltp)-Gene		Alary et al. 2002

6. Index of participant laboratories

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

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