DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 12/2014

DON and Zearalenone in Grains

Dienstleistung Lebensmittel Analytik GbR
Pinnberg 5
22927 Großhansdorf - Germany

proficiency-testing@dla-lvu.de **www.dla-lvu.de**

Coordinator: Dr. G. Wichmann

Contents

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed. 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 validity of the particular testing method.
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 principles of the DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009.

2. Realisation

2.1 Test material

The test material was a mixture of cornmeal and spelt flour with a natural content of DON and Zearalenon and 9% Glucose added for the homogeneity test. Approximately 3 kg of the material were homogenized and then packaged lightproof in portions to approximately 40 g. The portions were numbered chronologically. The material was checked for homogeneity.

2.1.1 Homogeneity

The calculation of the repeatability standard deviation of the participants for total-fumonisins was used additionally as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the official method E DIN EN 15891:2009-01 for corn flour (13). The repeatability standard deviation of the participants is given in the documentation.

To verify the homogeneity of the test material glucose was added before homogenisation. The homogeneity was examined with glucose/ HPLC.

Sample	**Glucose**		
1	8,94	g/100g	
2	8,95	g/100g	
3	9,00	g/100g	
4	9,06	g/100g	
5	9,06	g/100g	
Mean	**9,00**	g/100g	
Standard deviation	**0,06**	**0,64**	**%**

The homogeneity is considered verified with a standard deviation of < 1%.

In the documentation the portion numbers are graphically assigned to the results of DON. There is no trend recognizable in the results which could suggest inhomogeneity.

2.2 Test

Two portions of test material were sent to every participating laboratory in the 4th week of 2014. The testing method was optional. The tests should be finished at 7. March 2014 the latest.

2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples (by email). The finally calculated concentrations of DON and Zearalenon as average of duplicate determinations of both numbered samples was used for the statistical evaluation.
Queried and documented were single results, recovery and the used testing method.
All participants submitted at least one result.

3. Evaluation

3.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6).
The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S_x) was calculated (6).

3.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (significance level: 5%) (5). Detected outliers were stated for information only, when z-score simultaneously was < -2 or > 2.

3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is calculated according to the following equation.

$$\hat{\sigma} \ (\%) = 2^{(1-0,5 \log X)}$$

Out of this is calculated the target standard deviation in µg/kg

$$\hat{\sigma} = X * \hat{\sigma} \ (\%) / 100.$$

The target standard deviation according to Horwitz was used in this PT for DON.

For analytes with a content below 120 µg/kg after the evaluation of a lot of mycotoxin-proficiency testing schemes after 1997 Thompson suggested for the target standard deviation a steady value of 22 % (11), analogical

$$\hat{\sigma} = 0{,}22\ C / mr$$

with $\hat{\sigma}$ = Target standard deviation for contents < 120 µg/kg
C = measured value, expressed as a dimensionless mass ratio
mr = dimensionless mass ratio.

The target standard deviation according to Thompson (11) was used for analysing the results of Zearalenon.

3.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \quad .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated:

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

The precision data of the method E DIN EN 15891:2009-01 for a comparable DON content/corn (13) results in a relative target standard deviation of 21,3 % for DON and the German official method ASU §64 LFGB L 48.02-3 gave for a comparable Zearalenon content/corn a relative target standard deviation of 16,5 % for Zearalenon. This target standard deviation is given for information in the evaluation.

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 ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X)(6).

Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \ ;$$

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

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

3.6 Quotient $S^x/\hat{\sigma}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation 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.

For this PT the results show a sufficient comparability.

3.7 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty (u_X) for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x/\sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT (6). The quotient $u_X/\hat{\sigma}$ is reported in the characteristics of the test.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

In the upper table – test - the characteristics are listed:

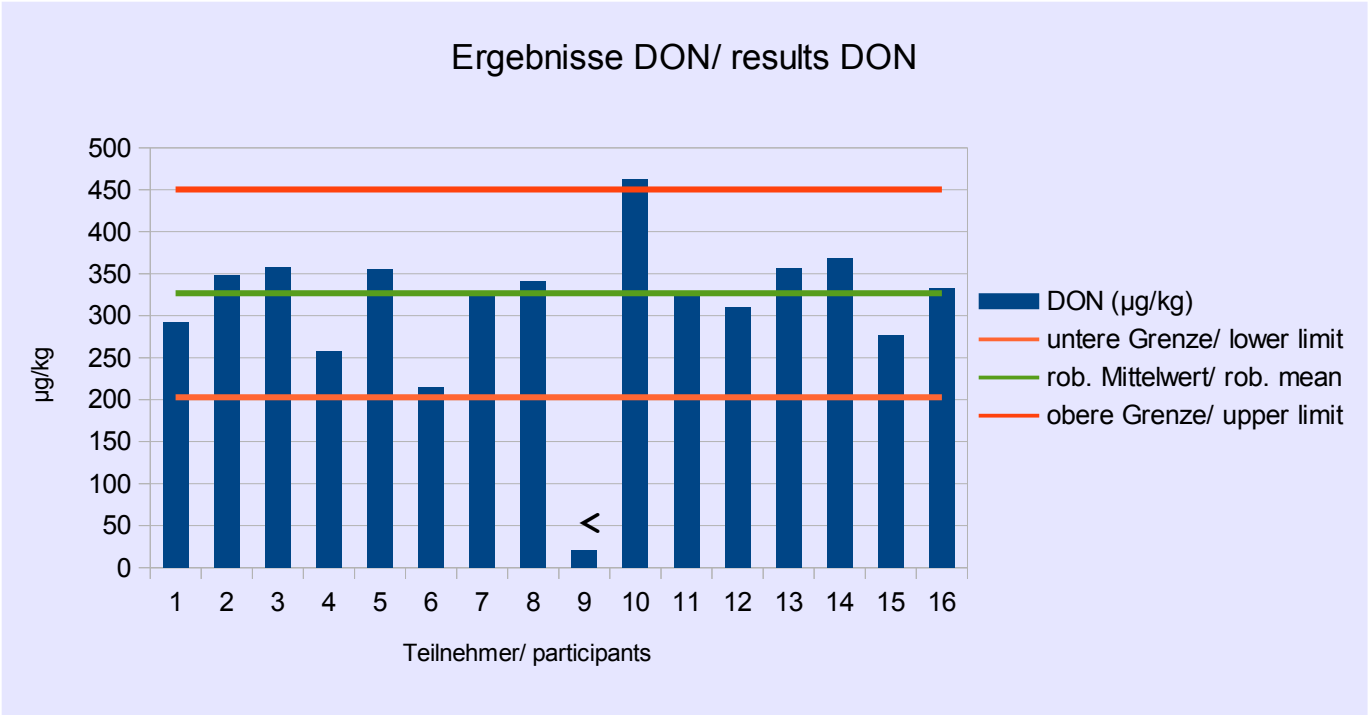
Number of results	
Number of outliers	
Mean	
Median	
Robust mean (X)	
Robust standard deviation (S^x)	
Target standard deviation($\hat{\sigma}$)(Horwitz/Thomson)	
Target standard deviation (E DIN EN 15891:2009-01 and ASU §64 LFGB L 48.02-3) for information	
Lower limit of target range (X - 2 $\hat{\sigma}$)	
Upper limit of target range (X + 2 $\hat{\sigma}$)	
Quotient $S^x/\hat{\sigma}$	
Standard uncertainty u_x	
Quotient $u_X/\hat{\sigma}$	
Number of results in the target range	

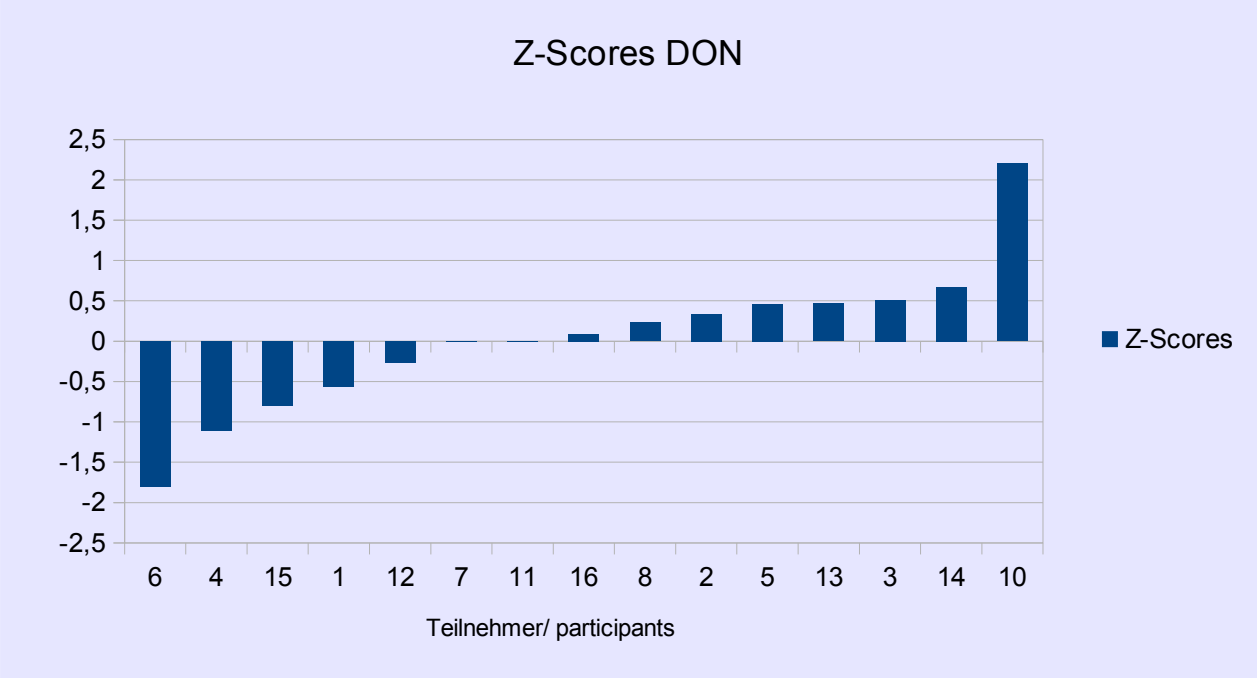
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

Evaluation number	Result	Deviation	z-Score	Remark

4.1 Deoxynivalenol in µg/kg

Characteristics	
Number of results (without no. 9)	15
Number of outliers	1
Mean	328
Median	333
Robust mean (X)	327
Robust standard deviation (S^x)	46
Target standard deviation($\hat{\sigma}$)	61,9
Target standard deviation (L 15.05-3 for information)	69,6
Lower limit of target range (X - 2 $\hat{\sigma}$)	203
Upper limit of target range (X + 2 $\hat{\sigma}$)	450
Quotient $S^x/\hat{\sigma}$	0,7
Standard uncertainty u_x	15,0
Quotient $u_X/\hat{\sigma}$	0,2
Number of results in the target range	14 (93%)



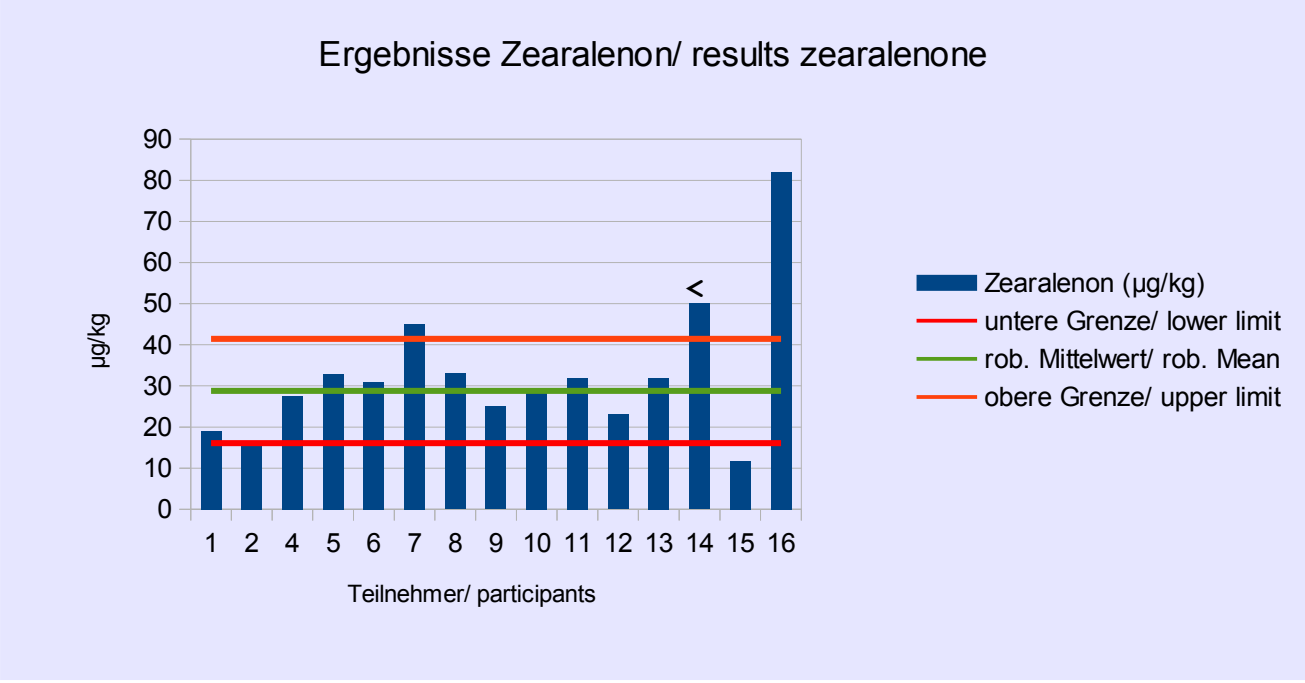


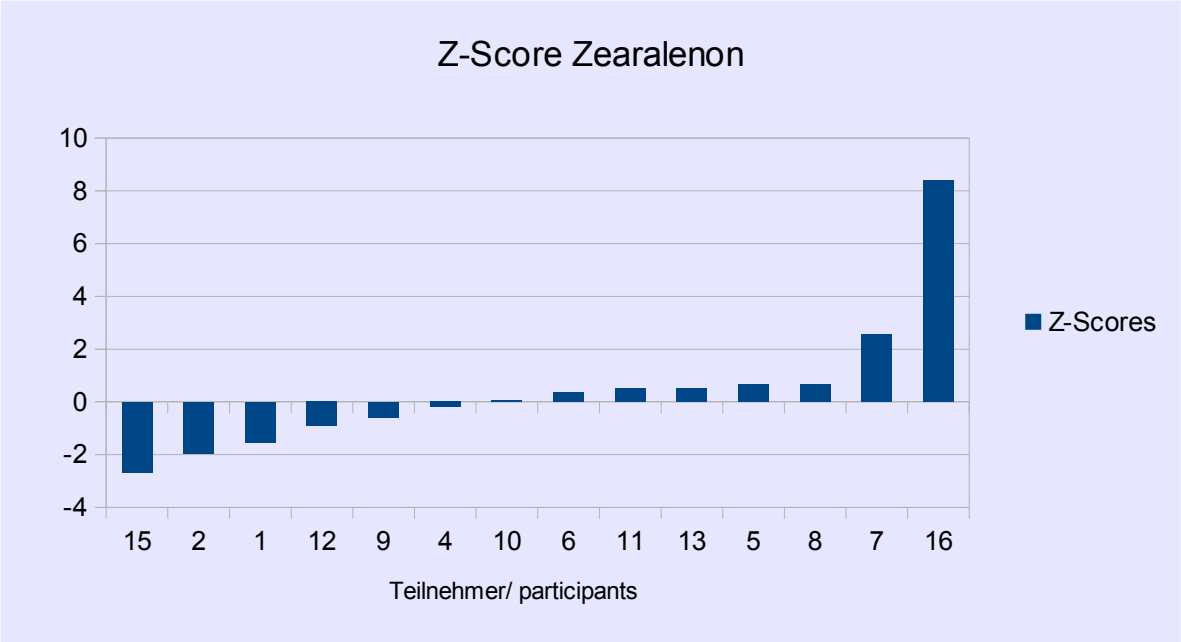
Laboratories

Teilnehmer/ participant	DON (µg/kg)	Abweichung/ deviation	z-Score s	Bemerkung/ remark
1	291,9	-34,83	-0,6	
2	348	21,27	0,3	
3	358	31,27	0,5	
4	258	-68,73	-1,1	
5	355	28,27	0,5	
6	214,81	-111,92	-1,8	
7	326	-0,73	0,0	
8	341	14,27	0,2	
9	< 20		**< 4,9**	**result/ LOD significantly below target range**
10	463	136,27	**2,2**	**Ausreißer/ outlier**
11	326	-0,73	0,0	
12	310	-16,73	-0,3	
13	356	29,27	0,5	
14	368	41,27	0,7	
15	277	-49,73	-0,8	
16	332,55	5,82	0,1	

4.2 Zearalenon in µg/kg

Characteristics	
Number of results (without no. 14)	14
Number of outliers	1
Mean	32,4
Median	30,0
Robust mean (X)	28,8
Robust standard deviation (S^x)	10,3
Target standard deviation($\hat{\sigma}$)	6,3
Target standard deviation (L 48.02-3 for information)	4,8
Lower limit of target range (X - 2 $\hat{\sigma}$)	16,1
Upper limit of target range (X + 2 $\hat{\sigma}$)	41,4
Quotient $S^x/\hat{\sigma}$	1,6
Standard uncertainty u_x	3,4
Quotient $u_X/\hat{\sigma}$	0,5
Number of results in the target range	11 (79%)





Laboratories

Teilnehmer/ participant	Zearalenon (µg/kg)	Abweichung/ deviation	Z-Scores	Bemerkungen/ remarks
1	18,99	-9,76	-1,5	
2	16,4	-12,35	-2,0	
4	27,5	-1,25	-0,2	
5	32,95	4,20	0,7	
6	30,98	2,23	0,4	
7	45	16,25	**2,6**	
8	33,0	4,25	0,7	
9	25	-3,75	-0,6	
10	29	0,25	0,0	
11	32	3,25	0,5	
12	23	-5,75	-0,9	
13	32	3,25	0,5	
14	< 50			LOD above target range
15	11,8	-16,95	**-2,7**	
16	81,95	53,20	**8,4**	Ausreißer/ outlier

5. Documentation

5.1 Primary data

5.1.1 DON in µg/kg

Teilnehmer/ participant	Ergebnis/ result	DLA No. A	DLA No. B	Ergebnis A/ result A	Ergebnis B/ result B	Wiederfindung/ recovery
	µg/kg			µg/kg	µg/kg	%
1	291,9	34	60	297,9	285,9	100
2	348	9	27	347	349	92
3	358	30	55	355	361	90
4	258	6	28	266	250	89
5	355	39	45	320	390	100
6	214,81	21	44			
7	326	65	48	318	334	83,5
8	341	50	71	340	342	109
9	<20	12	36	<20	<20	99
10	463	38	62	423	503	90
11	326	14	43	313	338	72
12	310	7	46	313,5	306,5	80
13	356	35	66	347	365	96,5
14	368	11	69			without recovery
15	277	3	42	282	272	87,6
16	332,55	20	49	317,5	347,5	

5.1.2 Zearalenon in µg/kg

Teilnehmer/ participat	Ergebnis/ result	DLA No A	DLA No B	Ergebnis A/ result A	Ergebnis B/ result B	Wiederfindung/ recovery
	µg/kg			µg/kg	µg/kg	%
1	18,99	34	60	18,4	19,58	98,2
2	16,4	9	27	16,8	16	108
3		30	55			
4	27,5	6	28	29,9	25	106
5	32,95	39	45	32,7	33,2	100
6	30,98	21	44			
7	45	65	48	41	50	76
8	33,0	50	71	30	36	99
9	25	12	36			99
10	29	38	62	26	32	100
11	32	14	43	26	39	101
12	23	7	46	23	23	
13	32	35	66	31	32	100
14	<50	11	69			without recovery
15	11,8	3	42	11,9	11,8	78,2
16	81,95	20	49	82,3	81,6	92

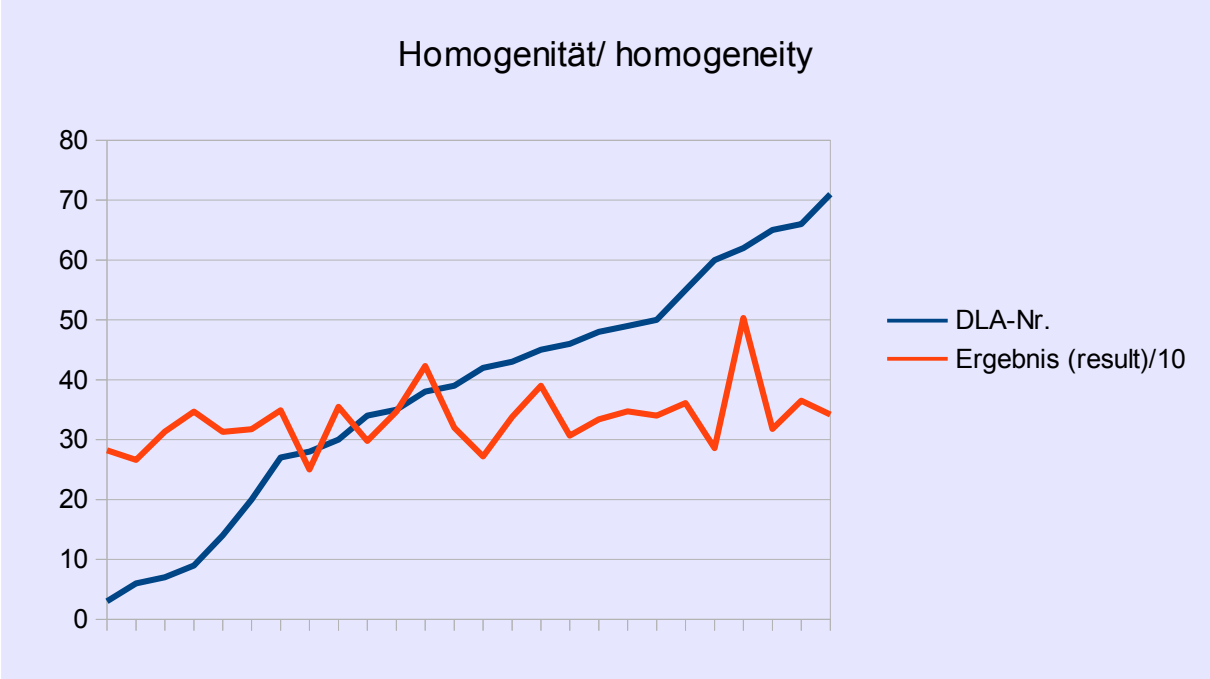
5.2 Homogeneity

5.2.1 Repeatability standard deviation of participants

The repeatability standard deviation of the single results was calculated for DON as described in chapter 5.1.1.
It is 32,8 µg/kg = 10,0 % of X (Deoxynivalenol).

5.2.2 Comparison of sample number/test result

The comparison of the increasing sample-numbers and measured total DON-results (/10) shows a sufficient homogeneity.



5.3 Analytical methods

5.3.1 Deoxynivalenol(DON)

Teilnehmer/ participant	Methode/ Method	Wiederfindung/ Recovery mit gleicher Matrix/ with same Matrix	Akkreditiert/ Accredited	Hinweise/ Remarks
1	-	yes	yes	-
2	Determination of Deoxynivalenol using PV 3076 (2011-06)	yes	yes	-
3	In house method LC-MS/MS	yes	yes	-
4	official collection of analysis methods §64 LFGB 0034	yes	yes	-
5	enzyme immunoassay (ELISA, r-biopharm)	yes	yes	-
6	Ridascreen Fast DON SC (Elisa)	yes	yes	-
7	-	yes	yes	-
8	BVL F 0034	yes	-	-
9	ELISA using Fa. R-biopharm	yes	yes	-
10	Deoxynivalenol: wet chemical extraction and clean up with IAC. Determination with HPLC/DAD; basic standard: for feedingstuff EN 15791, for food EN 15891	yes	yes	-
11	MS-120 (LC-MS/MS)	yes	yes	-
12	HPLC	no	yes	-
13	Extraction with ACN/H2O 80/20 (v/v); Determination with LC-MS/MS	yes	no	-
14	ELISA Fast-DON R-Biopharm	no	yes	-
15	HPLC	yes	yes	-
16	QSA-O-0680: Extraction with ACN/H2O (84/16; v/v); clean up of the extract with MycoSep-226-column; determination with LC-MS/MS (internal standard)	yes	yes	-

*IAC = Immuno Affinity Column

5.3.1 Zearalenon

Teilnehmer/ participant	Methode/ Method	Wiederfindung/ Recovery mit gleicher Matrix/ with same Matrix	Akkreditiert/ Accredited	Hinweise/ Remarks
1	-	yes	yes	-
2	Determination of Zearalenon using PV 3066 (2011-06)	yes	yes	-
3	-	-	-	-
4	HPLC-method with clean up using IAC VDLUFA method manual III 16.9.2	yes	yes	-
5	enzyme immunoassay (ELISA, r-biopharm)	yes	yes	-
6	Ridascreen Fast ZEA SC (Elisa)	-	yes	-
7	-	yes	no	-
8	BVL L 15.01-02.2	yes	yes	-
9	ELISA using Fa. R-biopharm	yes	yes	-
10	Zearalenon: wet chemical extraction and Clean Up with IAC. Determination with HPLC/FLD; Basic standard: for feedingstuff EN 15792, for food EN 15850	yes	yes	-
11	MS-120 (LC-MS/MS)	yes	-	-
12	HPLC	-	yes	-
13	Extraction with ACN/H2O 80/20 (v/v); Determination with LC-MS/MS	yes	no	-
14	ELISA Fast Zearalenon R-Biopharm	no	no	-
15	QSA-O-0680: Extraction with ACN/H2O (84/16; v/v); clean up of the extract with MycoSep-226-column; determination with LC-MS/MS (external standard)	yes	yes	-
16	HPLC-FLD	yes	yes	-

*IAC = Immuno Affinity Column

6. Index of participant laboratories

Teilnehmer/ paticipant	Ort/ location
	Austria
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Greece
	Deutschland
	Deutschland
	Switzerland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland

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

7. Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. 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
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
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13. E DIN EN 15891:2009-01 pr EN 15891:2009 (D), Foodstuffs – Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children – HPLC method with immunoaffinity column cleanup und UV-detection.
14. ASU §64 LFGB L 15.01/02-2: Bestimmung von Zearalenon in Weizen und Roggen (Dezember 2006)
15. ASU §64 LFGB L 16.01-8: Bestimmung von Zearalenon in Gerstenmehl, Maismehl und Weizenmehl (Januar 2011)
16. ASU §64 LFGB L 16.02-1: Bestimmung von Zearalenon in Maisgrieß (Januar 2011)
17. ASU §64 LFGB L 48.02-3: Bestimmung von Zearalenon in Säuglings- und Kleinkindernahrung (Januar 2011)

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