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Comparative Testing Report on the Detection and Quantification of Maize Event MON 810

Comparative testing round: ILC-CRL-GMFF-CT-02/10

D. Charels, T. Weber, M. Maras, M. Mazzara, C. Charles Delobel, C. Savini, G. Van den Eede

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Joint Research Centre
Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Report number: EURL-CT-02/10 CTRb
Status: recalled final report
Motivation: The uncertainty estimations depicted in Tables 1 and 2 of the final report have been corrected.
Confidentiality statement: The laboratory codes assigned to each participant in this comparative testing round are confidential. However, the EURL-GMFF will disclose details of the National Reference Laboratories that have been appointed under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006 to DG SANCO for the purpose of an assessment of their performance.

Executive Summary

The Joint Research Centre as European Union Reference Laboratory for Genetically Modified Food and Feed, established by Regulation (EC) No 1829/2003[1], organised a comparative testing round for National Reference Laboratories nominated under Regulation (EC) No 882/2004[2] and Regulation (EC) No 1981/2006[3] and for laboratories from third countries that volunteered to participate.

In accordance with Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for Genetically Modified Food and Feed shall organise comparative testing and shall ensure an appropriate follow-up of such testing.

The design and execution of the comparative testing round was in accordance with the ISO 17043 standard[4]. The European Union Reference Laboratory for Genetically Modified Food and Feed is in the process to become ISO 17043 accredited.

The test items used in the comparative testing round ILC-CRL-GMFF-CT-02/10 were produced by the Reference Materials Unit of the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). Participants had to determine the genetically modified (GM) content in two test items denoted maize powder levels 1 and 2, containing different GM percentages of maize event MON 810 flour. Maize powder levels 1 and 2 were 0.8 % and 3.8 % GM MON 810 flours that were produced under conditions defined in a previous interlaboratory study[5] . In June 2010, a total of 136 laboratories were invited to participate in ILC-CRL-GMFF-CT-02/10. Six National Reference Laboratories declined participation, of which two were no longer a National Reference Laboratory. One hundred and three laboratories registered for this comparative testing round. Test items were shipped to the participants beginning of September 2010 in dark brown glass bottles containing approximately 1 g of flour. Ninety laboratories from 41 countries returned results, of which:

1. 3 were National Reference Laboratories nominated only under Regulation (EC) No 882/2004
2. 31 were National Reference Laboratories nominated only under Regulation (EC) No 1981/2006,
3. 31 were National Reference Laboratories nominated under both Regulations,
4. 6 were members of the European Network of GMO Laboratories only and
5. 19 were laboratories from third countries

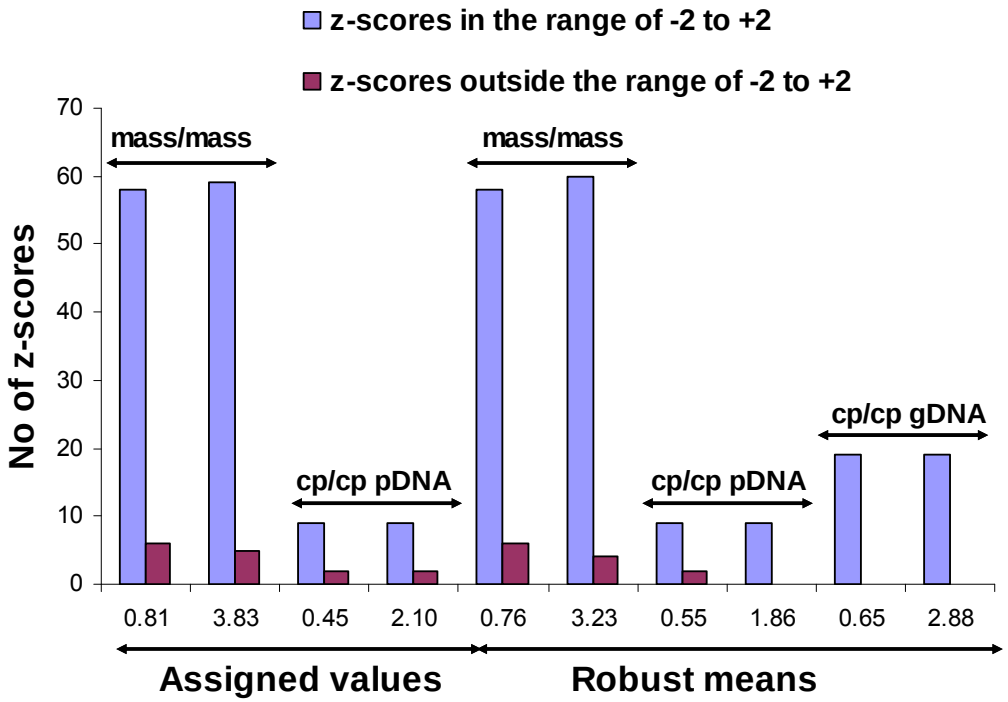
Four National Reference Laboratories submitted results in both measurement units. Thirteen laboratories including two National Reference Laboratories, two Official control laboratories and

nine laboratories from a third country did not submit any results. The Food Safety and Quality Unit of IRMM managed the on-line registration and submission of results.

Participants could report the results in either mass/mass % or copy/copy %. For the data expressed in mass/mass % the assigned values (μ) and associated uncertainties were provided by the Reference Materials Unit of IRMM. For maize powder levels 1 and 2, data from the homogeneity study conducted at the European Union Reference Laboratory for Genetically Modified Food and Feed's premises were included in the uncertainty budget. In addition, the European Union Reference Laboratory for Genetically Modified Food and Feed calculated the robust means ($\hat{\mu}$) of the maize powder levels 1 and 2 test items in mass/mass % and in copy/copy %. All data were log-transformed and then robust statistics were applied to obtain a robust mean [6, 7, 8].

The target standard deviation for comparative testing $\hat{\sigma}$ for maize event MON 810 was fixed to 0.2 ($\log_{10}$ value) by the Advisory Board for Comparative testing on the basis of the state-of-the-art in this field of analysis. This target standard deviation was used to derive z-scores for the participants' results. An overview of the assigned values, robust means and number of z-scores in the range of -2 to +2 is given in Figure 1.

Figure 1. Overview of z-scores calculated on the basis of assigned values and robust means, respectively. mass/mass = mass/mass %, cp/cp = copy/copy %



The outcome of this second comparative testing round was in general positive, with 82-100 % of participants gaining a z-score in the range of -2 to +2 for both maize powder levels 1 and 2 regardless of the calibration method, the measurement unit and the approach used for calculating the z-score.

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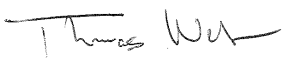
T. Weber (Sampling and statistics officer) 

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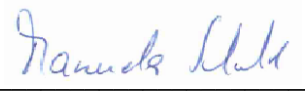
M. Schulze 

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1. Introduction

The Joint Research Centre (JRC) as European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) was established by Regulation (EC) No 1829/2003[(1)] of 22 September 2003 on genetically modified food and feed. The EURL-GMFF has two mandates determined by Regulation (EC) No 1981/2006[(3)] of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the Community reference laboratory for genetically modified organisms (GMOs) and by Regulation (EC) No 882/2004[(2)] of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

In accordance with Article 32 of Regulation (EC) No 882/2004 the EURL-GMFF shall organise comparative testing for National Reference Laboratories (NRLs) and shall ensure an appropriate follow-up of such testing. The aim of this activity is 'to contribute to a high quality and uniformity of analytical results'[(2)]. Moreover, Article 12 of Regulation (EC) No 882/2004 states that the nominated NRLs should be accredited in accordance with ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. One of the requirements of ISO/IEC 17025 accredited laboratories is to prove their competence by taking part in a proficiency testing scheme.

Regulation (EC) No 1829/2003 establishes a threshold for labelling of food and feed products consisting of or containing more than 0.9 % genetically modified organisms (GMOs) provided the GMO has undergone the authorisation procedure in accordance with European Union legislation. This threshold of 0.9 % for labelling is used by the Member States of the European Union involved in the official control of food and feed. Hence, a proper determination of the GM content in sampled products is of paramount importance.

The EURL-GMFF organised the second comparative testing round in 2010 in collaboration with the Reference Materials (RM) Unit and the Food Safety and Quality (FSQ) Unit of IRMM. The comparative testing round was announced at the ENGL meeting on the 19th and 20th of May 2010. In June 2010, a total of 136 laboratories were invited to participate in ILC-CRL-GMFF-CT-02/10. Six National Reference Laboratories declined participation, of which two were no longer a National Reference Laboratory. One hundred and three laboratories registered for this comparative testing round. Test items were shipped between the 6th and 8th of September 2010. The deadline for submission of results was the 22nd of October 2010. The FSQ Unit of IRMM managed the on-line registration and submission of results employing a database of the International Measurement Evaluation Programme (IMEP).
Ninety laboratories from 41 countries returned results, of which:

1. 3 were National Reference Laboratories nominated only under Regulation (EC) No 882/2004

2. 31 were National Reference Laboratories nominated only under Regulation (EC) No 1981/2006,
3. 31 were National Reference Laboratories nominated under both Regulations,
4. 6 were members of the European Network of GMO Laboratories only and
5. 19 were laboratories from third countries

Four National Reference Laboratories submitted results in both measurement units. Thirteen laboratories including two National Reference Laboratories, two Official control laboratories and nine laboratories from a third country did not submit any results.

2. Description of comparative test items

2.1 Preparation

Test items were prepared by the RM Unit of IRMM. The RM Unit produced test items for comparative testing according to ISO Guide 34[9] regarding the 'General requirements for the competence of reference material producers'.

Maize powder levels 1 and 2 were 0.8 % and 3.8 % GM MON 810 flours that were produced under conditions defined in a previous interlaboratory study[5].

Maize powders were prepared by a two-step grinding process using a high impact mill[10]. Test items were obtained by turbula-mixing and dry-mixing of non-modified maize powder and MON 810 maize powder. A 10 % GM mix was produced first using 100 % GM and non-GM base material. All lower concentrations were achieved by further dilution with non-GM maize powder. Powders were weighed using a calibrated balance.

Approximately 1 g of the dry-mixed test items were bottled in 10-mL brown glass vials using an automatic sampling device, under argon and re-labelled as maize powder levels 1 and 2. Test items were stored at +4 °C in the dark.

2.2 Homogeneity and stability assessment

The assessment of the homogeneity was performed after the test items had been packed in their final form and before distribution to participants[11].

Samples are considered to be adequately homogeneous if:

$$s_s \leq 0.3\hat{\sigma} \qquad (1)$$

Where s_s is the between-bottle standard deviation of samples as determined by a single factor ANOVA[12] and $\hat{\sigma}$ is the standard deviation for comparative testing.

If this criterion is met, the between-bottle standard deviation contributes no more than about 10 % to the standard deviation for comparative testing.
If this criterion is not met, the between-bottle standard deviation is included in the standard deviation for comparative testing:

$$\hat{\sigma}_1 = \sqrt{\hat{\sigma}^2 + s_S^2} \qquad (2)$$

The repeatability of the test method is the square root of mean sum of squares within bottles MS_{within}. The between-bottle standard deviation s_s is given by $\sqrt{MS_{between} - MS_{within}/n}$ where $MS_{between}$ is the mean sum of squares between bottles and n is the number of replicates. If $MS_{within} > MS_{between}$, then:

$$s_s = u^*_{bb} = \frac{repeatability}{\sqrt{n}} \sqrt[4]{\frac{2}{N(n-1)}} \qquad (3)$$

where u^*_{bb} is the maximum uncertainty contribution that can be obtained by the hidden heterogeneity of the material.
For each GM level ten brown glass vials (N = 10) were randomly selected and analysed in five-fold replicates (n = 5). The criterion described in formula (1) was fulfilled thus indicating that both maize powder test items were homogeneous.
The data from the homogeneity study conducted at the EURL-GMFF were used for the estimation of the uncertainty contributions related to the heterogeneity and to the stability of the maize powder levels 1 and 2 test items.

In order to test the stability of the test items used a t-test was carried out. A comparison of the arithmetic mean (N = 9) of the measurement results in this comparative testing round, omitting the outlying values, against the assigned values (0.47 cp/cp% versus 0.45 cp/cp% for level 1, respectively and 2.07 cp/cp % versus 2.10 cp/cp % for level 2, respectively) determined in a previous interlaboratory study[13] showed no significant differences (P = 0.96 and 0.82 for maize powder levels 1 and 2, respectively).

3. Participants' results

The assignment of a laboratory number to each participant and the submission of results were managed by the FSQ Unit of IRMM. Results had to be reported on-line using a form for which

each participant received an individual access code. A questionnaire was attached to the on-line reporting form to provide details of the analytical methods used.

Participants could report the results of the exercise in either mass/mass % (m/m %) or copy/copy % (cp/cp %). The expression of measurement results in cp/cp % follows the Recommendation (EC) No 2004/787[14], where it is recommended that the results of quantitative analyses are expressed as GM DNA copy numbers in relation to target taxon-specific copy numbers calculated in terms of haploid genomes.

Participants were instructed to apply the formulas described below when reporting their results.

$$\text{m/m \%} = \frac{\text{mass GM [g]}}{\text{Total mass [g]}} \times 100\ \% \qquad (4)$$

$$\text{cp/cp \%} = \frac{\text{GM DNA copy numbers [cp]}}{\text{Target taxon-specific DNA copy numbers [cp]}} \times 100\ \% \qquad (5)$$

A total of 90 laboratories from 41 countries reported results (Figure 2). Sixty-four laboratories reported the GM content in m/m %. Thirty laboratories expressed their results in cp/cp % of which 19 laboratories used a genomic and 11 laboratories used a plasmid DNA calibrant (Figure 3). Four laboratories reported the results in both measurement units. Thirteen laboratories including two NRLs, two Official control laboratories and nine laboratories from a third country did not submit any results. Both NRLs gave no reason for not reporting the results.

Figure 2: Distribution of participants from different countries

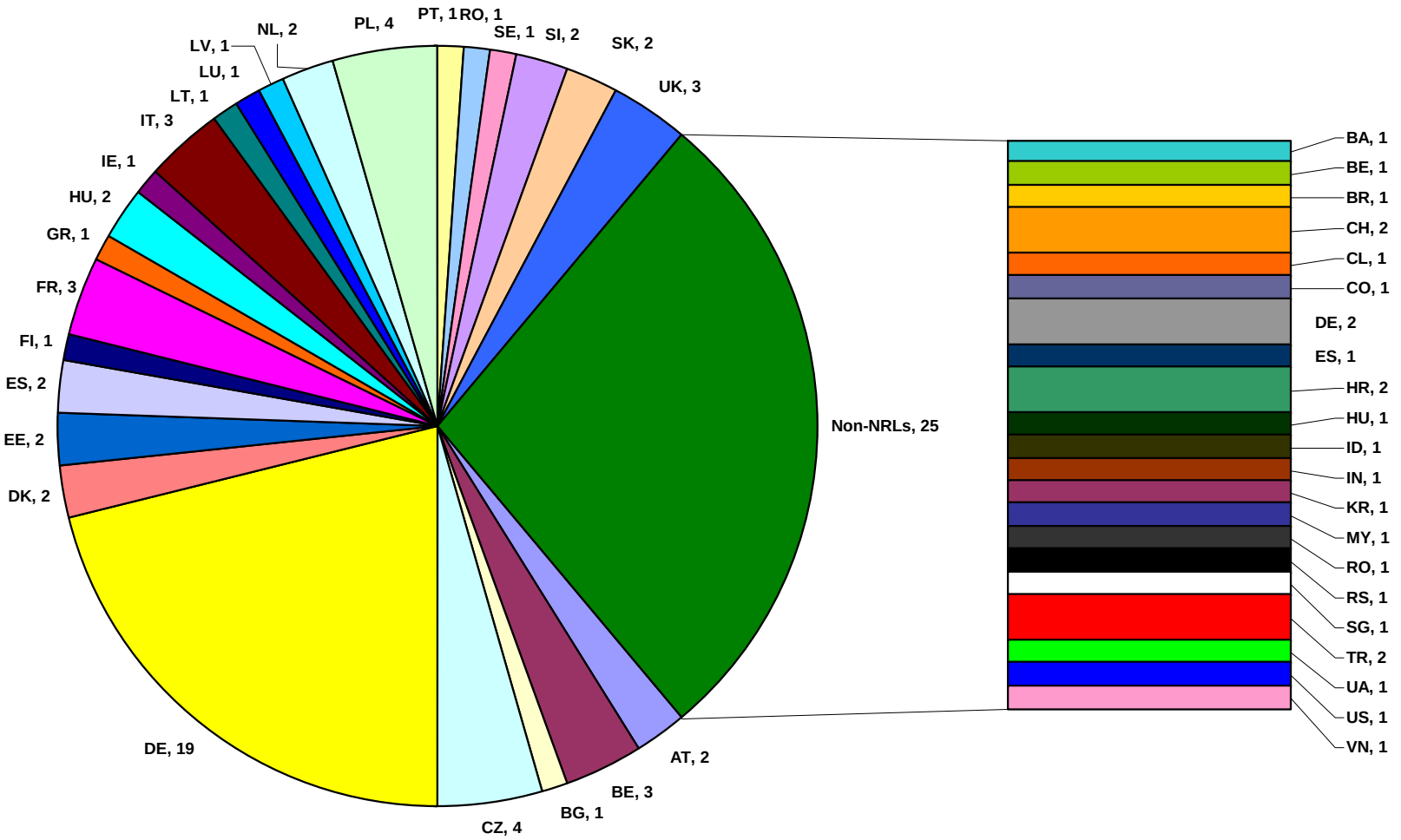
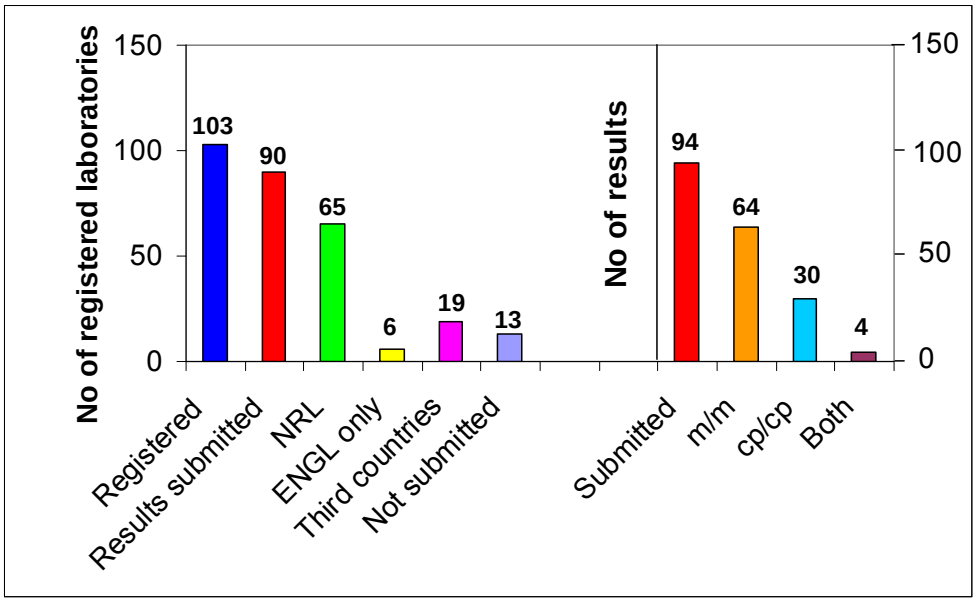


Figure 3. Overview of participants' results grouped per type of laboratory and measurement unit, respectively.



For the data expressed in m/m % and cp/cp % the assigned values (μ) and associated uncertainties were established during a previous interlaboratory study coordinated by IRMM[13]. Data from the homogeneity study conducted by the EURL-GMFF were also included in the uncertainty budget. In addition, the EURL-GMFF calculated the robust means ($\hat{\mu}$) of the maize powder levels 1 and 2 test items in m/m % and cp/cp %. All data were log-transformed and then robust statistics were applied to obtain a robust mean[6, 7, 8].

An overview of the results reported in m/m % and cp/cp % is given in Tables 3 to 6. An overview of the analytical methods used by each participant is summarised in the section on 'Questionnaire data'.

4. Assigned value and measurement uncertainty

4.1 Reference value determined by the test item producer

The assigned value in m/m % (μ), determined by the RM Unit of IRMM, is based on the mass fraction of non-GM and GM powder mixed and corrected for the water content[10]. The assigned value in cp/cp % (μ) is based on the use of a plasmid DNA calibrant[13].

The information relating to the CRL-GMFF-CT-02/10 maize powder levels 1 and 2 test items can be found in the table below.

Table 1. Assigned value μ and expanded uncertainty of maize powder levels 1 and 2

MON 810 maize content [m/m %]		Standard uncertainty[1]						Combined uncertainty	Expanded uncertainty
		$(u_1)^2$	$(u_2)^3$	$(u_3)^4$	$(u_4)^5$	$(u_5)^6$	$(u_6)^7$	(u_c)	$(U = 2 * u_c)$
Maize powder level 1[11]	0.81	0.00205	0.00004	0.02824	0.00289	0.00558	0.00888	0.03033	0.07
Maize powder level 2[11]	3.83	0.00813	0.00014	0.06503	0.00289	0.02646	0.04208	0.0823	0.17
MON 810 maize content [cp/cp %]		**Relative standard uncertainty contributions**						**Expanded uncertainty** $(U = 2 * u_c)$	
		$(u_{char,rel})^8$		$(u_{bb,rel})^9$		$(u_{lts,rel})^{10}$		U_{rel} [%]	U_{abs} [cp/cp %]
Maize powder level 1[12]	0.45	3.9		3.50		8.3		20	0.09
Maize powder level 2[12]	2.1	3.4		0.70		1.8		8	0.16

[1] Standard uncertainty contributions related to m/m % have been provided by IRMM
[2] Mass determination uncertainty introduced, mainly based on the uncertainty of the balance
[3] Water content measurement uncertainty, three and two dilution steps taken into consideration for maize powder levels 1 and 2, respectively.
[4] Standard uncertainty contribution resulting from the homogeneity assessment
[5] Purity of non-GM base material, based on the LOD of the method applied
[6] Purity of GM base material, based on the number of seeds tested individually
[7] Stability estimated to be 1.1 % relative u_{lts} for 12 months (based on comparable maize materials)
[8] Relative standard uncertainty relating to the characterisation(13).
[9] Relative standard uncertainty relating to the heterogeneity based on a sample intake of 100 mg.
[10] Relative standard uncertainty relating to the long-term stability, estimated on the basis of a shelf life of 12 months
[11] Assigned value in m/m %
[12] Assigned value in cp/cp %

The rounded certified values expressed in m/m % are: 0.81 +/- 0.07 % and 3.83 +/- 0.17 % for maize powder levels 1 and 2, respectively(15). The rounded certified values expressed in cp/cp % are: 0.45 +/- 0.09 % and 2.10 +/- 0.16 % for maize powder levels 1 and 2, respectively.

The expanded uncertainty of the certified value (U_{CRM}) comprises standard uncertainty contributions from the characterisation, the heterogeneity, and the stability(16).

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2} \qquad (6)$$

The combined standard uncertainty comprises contributions from the characterisation of the material (u_{char}), the between-vial heterogeneity (u_{bb}) at the recommended sample intake of 100 mg and the long-term stability of the material (u_{lts}). For the assigned value expressed in

m/m % the uncertainty contribution from the characterisation of the material includes uncertainties relating to the weighing procedure, the determination of the water content in the powders, and the purity of the non-GM and GM base materials (Table 1). A coverage factor of 2 was used to calculate the expanded uncertainty corresponding to a 95 % level of confidence[17].
The assigned values of maize powder levels 1 and 2 expressed in m/m % are traceable to the International System of Units (SI). The traceability chain is based on the use of calibrated balances and a thorough control of the weighing procedure.

4.2 Consensus value from participants

The consensus value ($\hat{\mu}$) from participants in the comparative testing round was calculated using robust statistics[18]. This approach minimises the influence of outlying values. All results were log-transformed prior to the calculation of the robust mean to establish a near-normal distribution allowing the interpretation of results on the basis of a normal distribution[7]. Two robust means ($\hat{\mu}$) were calculated on the basis of the results reported in m/m % and cp/cp %, respectively.

The uncertainty of the characterisation is assessed during the comparative testing round by estimating the relative standard deviation (RSD) of the robust mean. The standard uncertainty (u_{char}) of the characterisation is calculated using the formula:

$$u_{char} = \frac{RSD}{\sqrt{N}} \qquad (7)$$

where RSD = relative standard deviation of the robust mean and N = number of data points.

The value of the robust mean is traceable to the measurement unit of the reference material that was used for the preparation of the standard curves.

The assigned values (μ) by the test item producer and the robust means ($\hat{\mu}$) determined by the EURL-GMFF are depicted in Table 2.

Table 2. Overview of assigned values, robust means and expanded uncertainties for maize powder levels 1 and 2

Maize powder level 1	**[m/m %]**	U_{abs} [1] [m/m %]	**[cp/cp %]**	U_{abs} [1] [cp/cp %]
Assigned value	0.81	0.07	0.45[2]	0.09
Robust mean	0.76[3]	0.08	0.55[4]	0.17
			0.65[5]	0.15
Maize powder level 2				
Assigned value	3.83	0.17	2.10[2]	0.16
Robust mean	3.23[3]	0.19	1.86[4]	0.41
			2.88[5]	0.53

[1] *U* refers to an expanded uncertainty with a coverage factor *k* equal to 2 corresponding to a level of confidence of 95 %[17]
[2] Assigned value obtained with a plasmid DNA calibrant on the basis of $N = 30$[13]
[3] Robust mean calculated on the basis of $N = 64$.
[4] Robust mean obtained with a plasmid DNA calibrant and calculated on the basis of $N = 11$.
[5] Robust mean obtained with a genomic DNA calibrant and calculated on the basis of $N = 19$.

5. Statistical data and summaries

The aim of a performance statistic is to provide participants with a meaningful result that can be easily interpreted. The procedure followed for the evaluation of participants' performance was agreed by the Members of the Advisory Board and relies on the calculation of z-scores on the basis of the assigned value by the test item provider and the robust mean of the participants' results [11].

Laboratories are compared on the basis of z-scores calculated from log-transformed data[7]. Two types of z-scores are used, one based on the assigned value (μ) of the test item and the other based on the robust mean ($\hat{\mu}$) of the submitted results. Results reported in m/m % and results reported in cp/cp % using a plasmid DNA calibrant are analysed using both types of z-scores. For results reported in cp/cp % using a genomic DNA calibrant, only the robust mean is used to calculate a z-score.

The value of $\hat{\sigma}$, the target standard deviation for comparative testing, determines the performance limits in a comparative test and is set at a value that reflects best practice for the analysis in question. For this round the Members of the Advisory Board chose a value of 0.2[19]. The z-score (z_i) for participant i reporting measurement result x_i is thus calculated as

$$z_i = \left(\log_{10} x_i - \mu \right) \Big/ \hat{\sigma} \text{ or as } z_i = \left(\log_{10} x_i - \hat{\mu} \right) \Big/ \hat{\sigma} \qquad (8)$$

Table 3. Reported results (m/m %) and z-scores for event MON 810 maize powder level 1

Laboratory number	Maize event MON 810					
	Assigned value = 0.81 m/m % Robust mean = 0.76 m/m %					
	Value	uncertainty	LOD m/m	LOQ m/m	z-score[1]	z-score[2]
L004	0.76	0.12[c]	0.05	0.1	-0.14	0.03
L007	0.58	0.15[a]	-	-	-0.73	-0.56
L008	0.88	0.30[a]	-	-	0.18	0.35
L009	0.59	0.43[c]	-	-	-0.69	-0.52
L012	0.85	0.10[a]	-	0.1	0.10	0.27
L014	0.73	0.12[b]	0.1	0.1	-0.23	-0.06
L015	0.80	0.40[b,c]	-	-	-0.03	0.14
L016	1.41	0.09[c]	0.1	0.1	1.20	1.37
L017	1.07	0.00[c]	0.05	0.1	0.60	0.77
L020	3.53	0.31[c]	0.03	0,1	3.20	3.36
L022	0.65	0.23[a,c]	0.01	0.1	-0.48	-0.31
L023	0.74	0.22[b]	0.02	0.1	-0.20	-0.03
L024	0.54	0.04[a]	0	0.1	-0.88	-0.71
L027	0.53	0.35[b,c]	0.1	0.1	-0.92	-0.75
L028	0.80	0.08[a]	0.03	0.1	-0.03	0.14
L030	0.79	0.00[c]	0.01	0.03	-0.05	0.11
L031	2.90	0.10[c]	0.1	0.1	2.77	2.94
L032	0.95	0.29[a,c]	0.08	0.14	0.35	0.51
L033	0.56	0.17[a]	0.08	0.1	-0.80	-0.63
L034	0.77	0.50[a,c]	0.01	0.1	-0.11	0.06
L035	0.76	0.14[a,c]	0.02	0.1	-0.14	0.03
L037	0.47	0.06[c]	0.1	0.1	-1.18	-1.01
L038	0.50	31.00[b]	0.05	0.1	-1.05	-0.88
L039	0.80	0.20[b,c]	0.008	0.2	-0.03	0.14
L041	2.82	18.00[a,c]	0.05	0.1	2.71	2.88
L042	0.76	0.15[a]	0.025	0.05	-0.14	0.03
L043	0.62	0.06[a]	0.01	0.1	-0.58	-0.41
L045	0.66	0.11[b]	-	-	-0.44	-0.28
L046	0.90	0.27[a]	0.05	0.1	0.23	0.40
L047	0.78	0.12[a,c]	< 0.1	< 0.1	-0.08	0.09
L048	0.72	0.30[b,c]	-	-	-0.26	-0.09
L049	0.62	0.27[a]	0.011	0.11	-0.58	-0.41
L051	0.86	20.00[c]	0.05	0.1	0.13	0.30
L052	0.70	0.08[a]	0.03	0.06	-0.32	-0.15
L053	0.70	0.11[a]	< 0.1	0.1	-0.33	-0.16
L055	0.88	0.22[b]	0.05	0.1-5.0	0.18	0.35
L057	0.75	19.27[b,c]	0.01	0.1	-0.17	0.00
L060	3.80	0.10[b]	0.1	0.1	3.36	3.52
L061	1.04	0.00[c]	0.01	0.09	0.54	0.71
L064	0.50	0.14[a]	0.01	0.1	-1.07	-0.90
L065	0.57	100.00[b]	0.1	0.1	-0.76	-0.60
L067	0.80	0.20[a]	0.01	0.1	-0.03	0.14
L068	0.90	0.30[a]	0.01	0.1	0.23	0.40
L069	0.29	0.18[a,c]	0.05	0.1	-2.27	-2.10
L073	0.60	0.02[c]	0.02	-	-0.65	-0.48
L074	0.52	0.07[b,c]	-	-	-0.96	-0.79
L075	0.69	0.27[b,c]	0.04	0.13	-0.35	-0.18
L076	0.61	0.26[a,c]	0.01	0.1	-0.62	-0.45
L077	0.74	0.12[a,c]	<0.1	0.1	-0.20	-0.03
L078	1.02	0.09[a]	0.03	0.07	0.50	0.67
L081	1.10	42.00[c]	-	-	0.66	0.83
L083	0.48	0.35[a,c]	0.02	0.08	-1.14	-0.97
L085	0.80	0.10[b]	0.01	0.1	-0.03	0.14
L088	0.75	0.06[a]	0.02	0.1	-0.17	0.00
L089	1.19	0.78[a,c]	< 0.02	0.02	0.84	1.00
L090	0.72	0.07[a]	0.02	0.1	-0.26	-0.09
L093	0.81	0.08[a]	0.03	0.1	0.00	0.17
L094	0.23	0.21[a,c]	0.05	0.1	-2.73	-2.57
L095	0.70	0.00[c]	<0.1	0.1	-0.32	-0.15
L097	0.73	50.00[b,c]	<0.1	0.1	-0.23	-0.06
L099	0.96	0.02[c]	0.02	0.04	0.37	0.54
L101	0.85	0.06[b,c]	0.03	0.09	0.10	0.27
L103	0.65	0.23[a]	0.1	0.1	-0.49	-0.33
L105	1.00	20.00[c]	0.1	0.1	0.46	0.63

[a] Uncertainty reported as an absolute value, [b] Uncertainty reported as a relative value, [c] Inconsistent or incomplete information, [1] z-score calculated on the basis of the assigned value,[2] z-score calculated on the basis of the robust mean, LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, Results are as submitted by participants

Table 4. Reported results (cp/cp %) and z-scores for event MON 810 maize powder level 1 using a genomic DNA calibrant (4a) and a plasmid DNA calibrant (4b)

4a

Laboratory number	Maize event MON 810				
	Genomic DNA calibrant				
	Robust mean = 0.65 cp/cp %				
	value	**uncertainty**	**LOD**	**LOQ**	**z-score**
L001	**0.80**	9.00[a]	0.06	0.06	**0.48**
L002	**0.69**	0.00[c]	0.1	0.1	**0.16**
L003	**0.69**	0.00[c]	0.005	0.04	**0.14**
L013	**0.35**	0.09[b,c]	0.01	0.1	**-1.31**
L026	**0.66**	0.14[b,c]	0.1	0.1	**0.06**
L029	**0.49**	0.05[a,c, d]	-	-	**-0.58**
L040	**0.90**	0.17[a,c]	-	-	**0.74**
L047	**0.69**	0.10[a]	5 cp	10 cp	**0.16**
L050	**0.75**	0.26[b,c]	0.02	0.2	**0.34**
L055	**0.37**	0.09[b]	0.04	0.04	**-1.19**
L056	**0.76**	7.57[b,c]	0.011	0.105	**0.37**
L058	**0.54**	0.20[b,c]	0.02	0.1	**-0.37**
L059	**0.30**	0.10[b]	0.03	0.1	**-1.65**
L062	**0.64**	0.24[b,c]	25	55	**0.00**
L063	**0.62**	0.19[a]	0.5	0.5	**-0.07**
L070	**0.73**	25.00[c]	0.05	0.1	**0.28**
L096	**0.95**	0.41[a]	0.06	0.09	**0.85**
L098	**0.93**	0.20[a]	0.1	0.1	**0.81**
L104	**0.51**	0.12[b,c]	0	0.1	**-0.50**

4b

Laboratory number	Maize event MON 810					
	Plasmid DNA calibrant					
	Assigned value = 0.45 cp/cp %					
	Robust mean = 0.55 cp/cp %					
	value	**uncertainty**	**LOD**	**LOQ**	**z-score[1]**	**z-score[2]**
L005	**3.65**	0.23[a]	-	-	**4.11**	**4.20**
L021	**0.8**	0.20[a,c]	-	-	**0.81**	**0.91**
L025	**0.47**	0.15[b]	0.01	0.01	**0.09**	**-0.25**
L045	**0.48**	0.11[b]	-	-	**0.14**	**-0.20**
L066	**0.48**	0.21[a,c]	0.07	0.07	**0.14**	**-0.20**
L071	**0.44**	0.04[b,c]	0.04	0.08	**-0.05**	**-0.39**
L079	**0.44**	0.32[a,c]	0.005	-	**-0.05**	**-0.39**
L080	**0.45**	0.07[a,c]	<0.1	0.1	**0.00**	**-0.34**
L082	**0.4**	0.18[b,c]	0.01	0.01	**-0.26**	**-0.60**
L084	**0.29**	0.08[a]	0.05	0.1	**-0.95**	**-1.29**
L091	**3.16**	1.07[a,c]	-	-	**3.80**	**3.89**

[a] Uncertainty reported as an absolute value, [b] Uncertainty reported as a relative value, [c] Inconsistent or incomplete information, [d] Value obtained using digital PCR, [1] z-score calculated on the basis of the assigned value,[2] z-score calculated on the basis of the robust mean, LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, Results are as submitted by participants

Table 5. Reported results (m/m %) and z-scores for event MON 810 maize powder level 2

Maize event MON 810

Laboratory number	Assigned value = 3.83 m/m % Robust mean = 3.23 m/m %					
	Value	uncertainty	LOD m/m	LOQ m/m	z-score[1]	z-score[2]
L004	3.00	0.47[c]	0.05	0.1	-0.53	-0.14
L007	2.83	0.52[a]	-	-	-0.66	-0.27
L008	4.04	1.40[a]	-	-	0.12	0.51
L009	3.31	2.42[c]	-	-	-0.32	0.07
L012	3.87	0.54[a]		0.1	0.02	0.41
L014	3.38	0.37[b]	0.1	0.1	-0.27	0.12
L015	3.78	1.90[b,c]	-	-	-0.03	0.36
L016	0.99	0.07[c]	-	-	-2.94	-2.55
L017	4.94	0.00[c]	0.05	0.1	0.55	0.94
L020	0.77	0.10[c]	0,03	0,1	-3.48	-3.09
L022	2.76	0.24[a,c]	0.01	0.1	-0.71	-0.32
L023	3.47	1.04[b]	0.02	0.1	-0.21	0.18
L024	2.90	0.21[a]	0.01	0.1	-0.60	-0.21
L027	3.10	2.02[b,c]	0.1	0.1	-0.46	-0.07
L028	3.78	0.28[a]	0.03	0.1	-0.03	0.36
L030	3.90	0.00[c]	0.01	0.03	0.04	0.43
L031	0.70	0.10[c]	0.1	0.1	-3.69	-3.30
L032	4.04	1.15[a,c]	0.07	0.13	0.12	0.51
L033	1.83	0.55[a]	0.08	0.1	-1.60	-1.21
L034	3.70	2.40[a,c]	0.01	0.1	-0.07	0.31
L035	3.47	0.56[a,c]	0.02	0.1	-0.21	0.18
L037	2.41	0.30[c]	0.1	0.1	-1.01	-0.62
L038	3.08	31.00[b]	0.05	0.1	-0.47	-0.08
L039	4.20	1.30[b,c]	0.01	0.25	0.20	0.59
L041	0.92	18.00[a,c]	0.05	0.1	-3.10	-2.71
L042	3.50	0.50[a]	-	-	-0.20	0.19
L043	3.11	0.66[a]	0.01	0.1	-0.45	-0.06
L045	3.12	0.38[b]	-	-	-0.45	-0.06
L046	3.60	0.61[a]	0.05	0.1	-0.13	0.25
L047	3.54	0.94[a,c]	< 0.1	< 0.1	-0.17	0.22
L048	2.73	0.78[b,c]	-	-	-0.74	-0.35
L049	2.87	1.21[a]	0.013	0.13	-0.63	-0.24
L051	3.59	20.00[c]	0.05	0.1	-0.14	0.25
L052	3.48	0.25[a]	0.03	0.06	-0.21	0.18
L053	3.29	0.07[a]	< 0.1	0.1	-0.33	0.06
L055	3.82	0.76[b]	0.05	0.1-5.0	-0.01	0.38
L057	3.55	19.27[b,c]	0.01	0.1	-0.16	0.22
L060	2.90	0.30[b]	0.1	0.1	-0.60	-0.21
L061	3.38	0.00[c]	0.01	0.09	-0.27	0.12
L064	2.01	0.144[a]	0.01	0.1	-1.40	-1.01
L065	2.81	100.00[b]	0.1	0.1	-0.67	-0.28
L067	3.00	0.90[a]	0.01	0.1	-0.53	-0.14
L068	3.50	1.00[a]	0.01	0.1	-0.20	0.19
L069	1.35	0.87[a,c]	0.05	0.1	-2.26	-1.87
L073	2.80	0.28[c]	0.36	-	-0.68	-0.29
L074	2.50	0.28[b,c]	-	-	-0.93	-0.54
L075	3.10	0.27[b,c]	0.04	0.13	-0.46	-0.07
L076	3.56	1.52[a,c]	0.01	0.1	-0.16	0.23
L077	3.10	0.46[a,c]	<0.1	0.1	-0.46	-0.07
L078	3.19	0.06[a]	0.03	0.05	-0.40	-0.01
L081	5.60	42.00[c]	-	-	0.82	1.21
L083	2.14	1.55[a,c]	0.02	0.08	-1.26	-0.87
L085	3.80	0.50[b]	0.01	0.1	-0.02	0.37
L088	3.39	0.36[a]	0.02	0.1	-0.26	0.12
L089	4.88	3.20[a,c]	< 0.02	0.02	0.53	0.92
L090	3.73	0.22[a]	0.02	0.1	-0.06	0.33
L093	3.25	0.28[a]	0.03	0.1	-0.36	0.03
L094	1.56	0.86[a,c]	0.05	0.1	-1.95	-1.56
L095	3.80	0.00[c]	<0.1	0.1	-0.02	0.37
L097	3.12	50.00[b,c]	<0.1	0.1	-0.45	-0.06
L099	3.39	0.02[c]	0.02	0.04	-0.26	0.12
L101	3.16	0.08[b,c]	0.03	0.09	-0.42	-0.03
L103	2.85	1.02[a]	0.1	0.1	-0.64	-0.25
L105	8.00	9.00[c]	0.1	0.1	1.60	1.99

[a] Uncertainty reported as an absolute value, [b] Uncertainty reported as a relative value, [c] Inconsistent or incomplete information, [1] z-score calculated on the basis of the assigned value, [2] z-score calculated on the basis of the robust mean, LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, Results are as submitted by participants

Table 6. Reported results (cp/cp %) and z-scores for event MON 810 maize powder level 2 using a genomic DNA calibrant (6a) and a plasmid DNA calibrant (6b)

6a

Maize event MON 810

Genomic DNA calibrant

Robust mean = 2.88 cp/cp %

Laboratory number	value	uncertainty	LOD	LOQ	z-score
L001	**3.30**	9.00[a]	0.06	0.06	**0.58**
L002	**3.47**	0.00[c]	0.1	0.1	**0.69**
L003	**4.50**	0.00[c]	0.005	0.04	**1.26**
L013	**1.34**	0.20[b,c]	0.01	0.1	**-1.37**
L026	**3.86**	1.63[b,c]	0.1	0.1	**0.92**
L029	**2.26**	0.30[a,c, d]	-	-	**-0.24**
L040	**3.24**	0.62[a,c]	-	-	**0.54**
L047	**2.31**	0.62[a]	5 cp	10 cp	**-0.19**
L050	**3.00**	0.60[b,c]	0.02	0.2	**0.38**
L055	**1.44**	0.29[b]	0.04	0.04	**-1.22**
L056	**3.30**	11.38[b,c]	0.01	0.101	**0.58**
L058	**2.04**	0.70[b,c]	0.02	0.1	**-0.46**
L059	**1.30**	0.20[b]	0.03	0.1	**-1.44**
L062	**3.03**	1.08[b,c]	25	55	**0.40**
L063	**4.74**	1.42[a]	0.5	0.5	**1.37**
L070	**4.00**	25.00[c]	0.05	0.1	**1.00**
L096	**3.45**	0.58[a]	0.06	0.09	**0.68**
L098	**2.31**	0.31[a]	0.1	0.1	**-0.19**
L104	**2.10**	0.21[b,c]	0.0%	0.1%	**-0.40**

6b

Maize event MON 810

Plasmid DNA calibrant

Assigned value = 2.10 cp/cp %

Robust mean = 1.86 cp/cp %

Laboratory number	value	uncertainty	LOD	LOQ	z-score[1]	z-score[2]
L005	**0.76**	0.14[a]	-	-	**-2.21**	**-1.91**
L021	**2.8**	0.70[a,c]	-	-	**0.62**	**0.92**
L025	**2.10**	0.50[b]	0.01	0.03	**0.00**	**0.11**
L045	**2.23**	0.46[b]	-	-	**0.13**	**0.24**
L066	**2.29**	1.03[a,c]	0.07	0.07	**0.19**	**0.30**
L071	**1.92**	0.19[b,c]	0.0%	0.1%	**-0.19**	**-0.08**
L079	**2.24**	1.62[a,c]	0.01		**0.14**	**0.25**
L080	**1.86**	0.28[a,c]	<0.1	0.1	**-0.26**	**-0.15**
L082	**1.77**	0.79[b,c]	0.01	0.01	**-0.37**	**-0.26**
L084	**1.41**	0.25[a]	0.05	0.1	**-0.87**	**-0.75**
L091	**0.79**	0.17[a,c]	-	-	**-2.12**	**-1.83**

[a] Uncertainty reported as an absolute value, [b] Uncertainty reported as a relative value, [c] Inconsistent or incomplete information, [d] Value obtained using digital PCR, [1] z-score calculated on the basis of the assigned value,[2] z-score calculated on the basis of the robust mean, LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, Results are as submitted by participants

Figure 4. z-scores for maize event MON 810 powder level 1 on the basis of an assigned value of 0.81 m/m % (□) and a robust mean of 0.76 m/m % (◊)

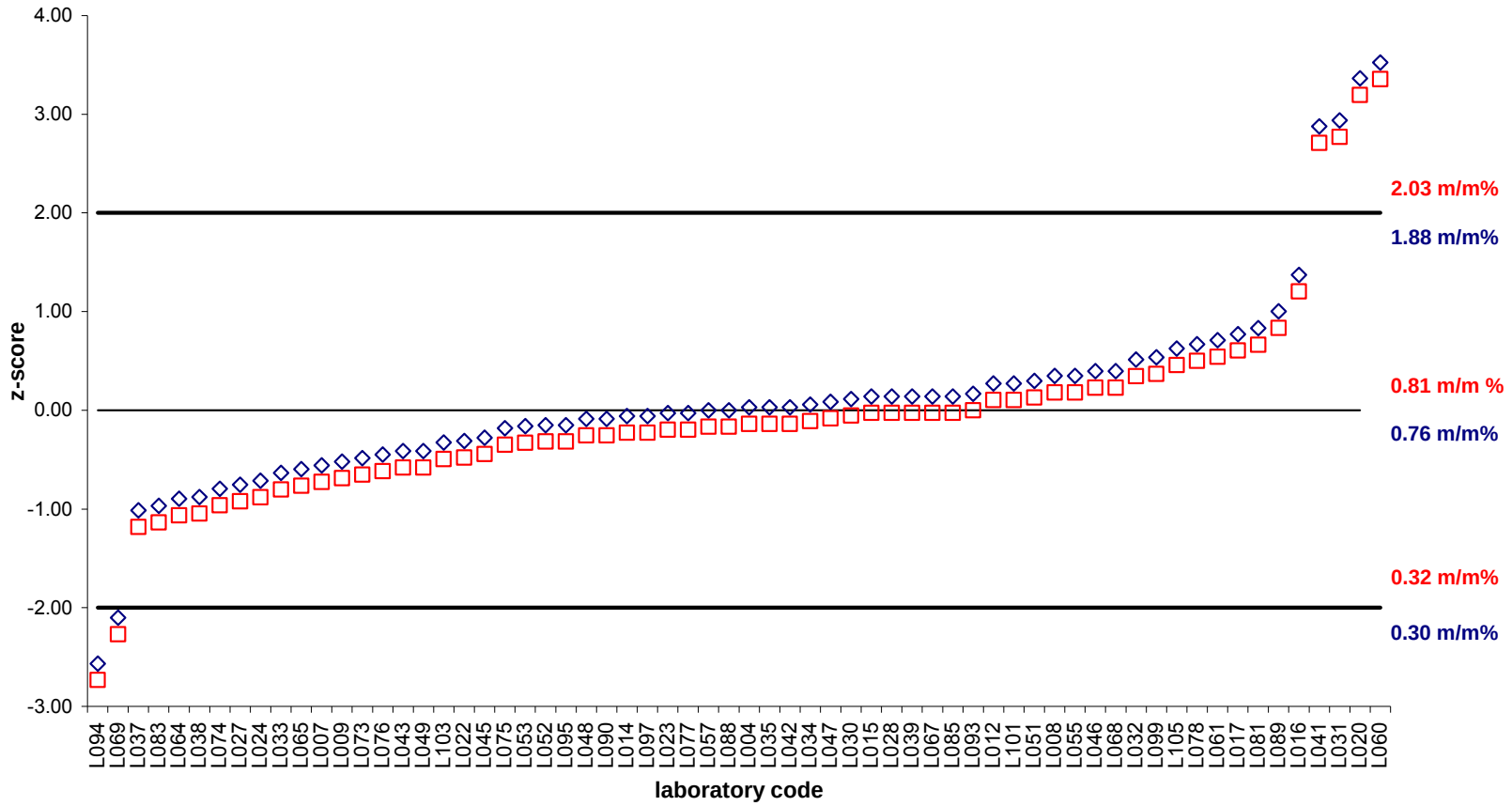


Figure 5. z-scores for maize event MON 810 maize powder level 1 on the basis of a robust mean of 0.65 cp/cp % obtained with a genomic DNA calibrant

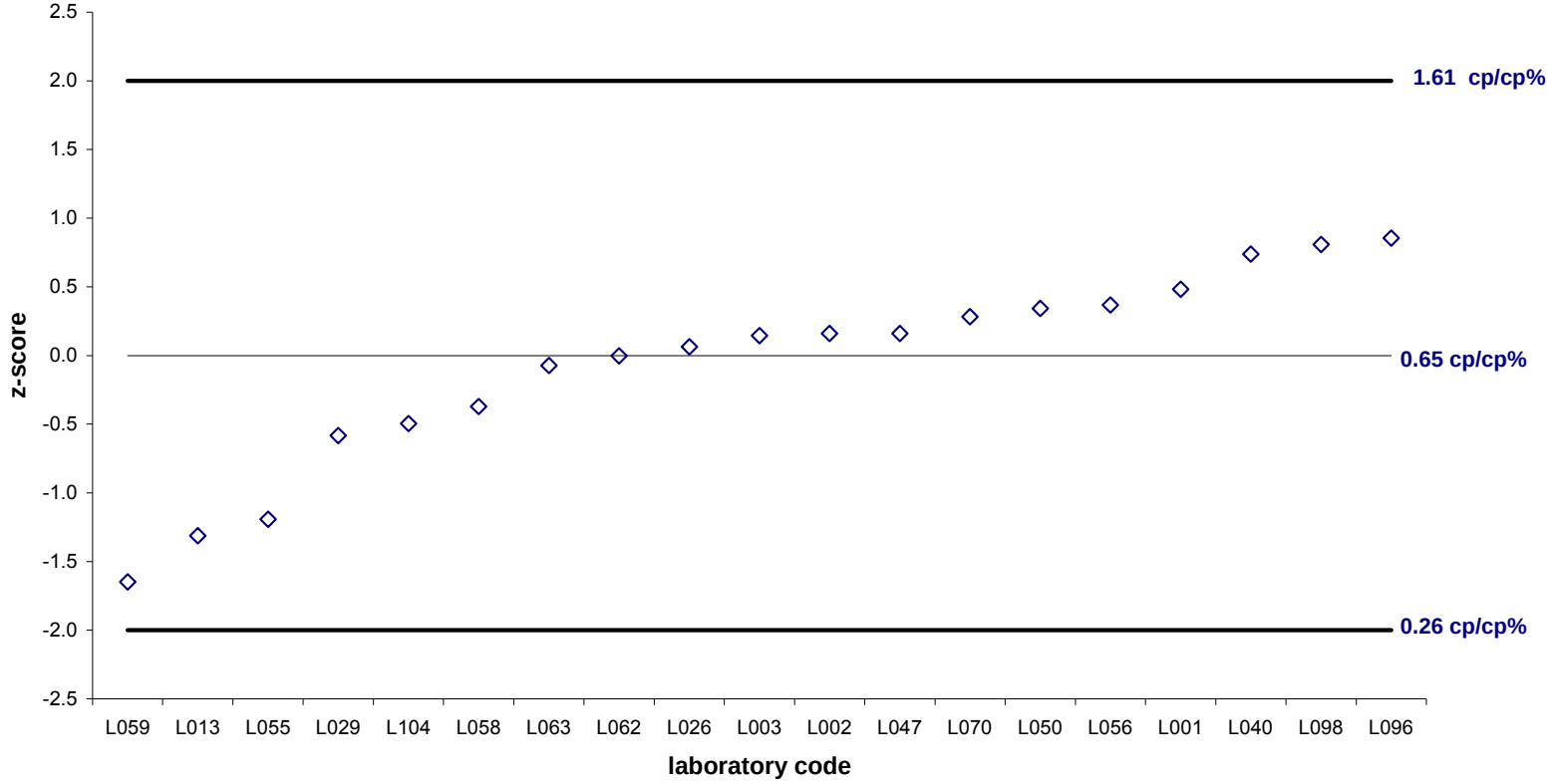


Figure 6. z-scores for maize event MON 810 powder level 1 on the basis of an assigned value of 0.45 cp/cp % (□) and a robust mean of 0.55 cp/cp % (◊) obtained with a plasmid DNA calibrant

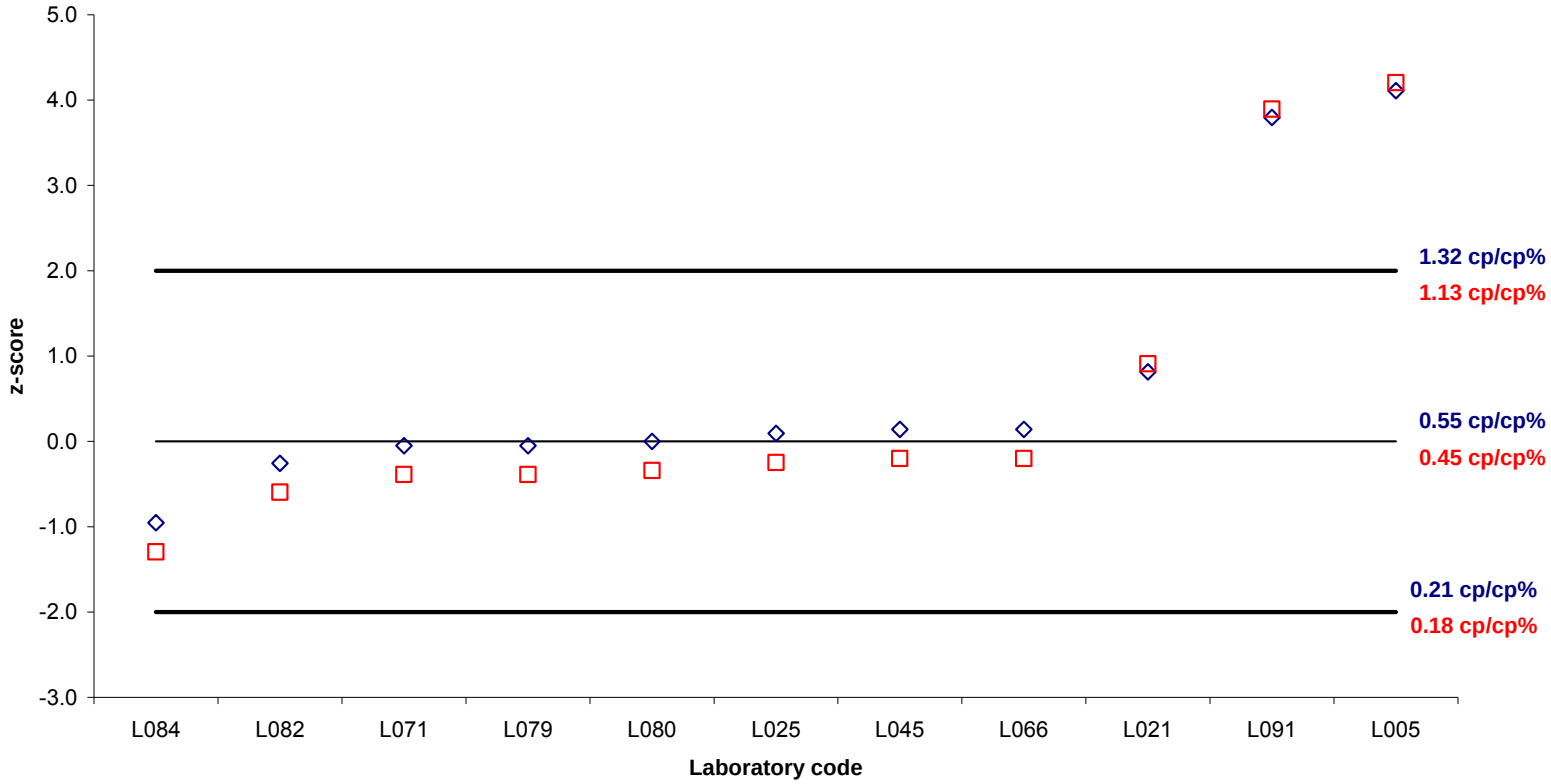


Figure 7. z-scores for maize event MON 810 powder level 2 on the basis of an assigned value of 3.83 m/m % (□) and a robust mean of 3.23 m/m % (◊)

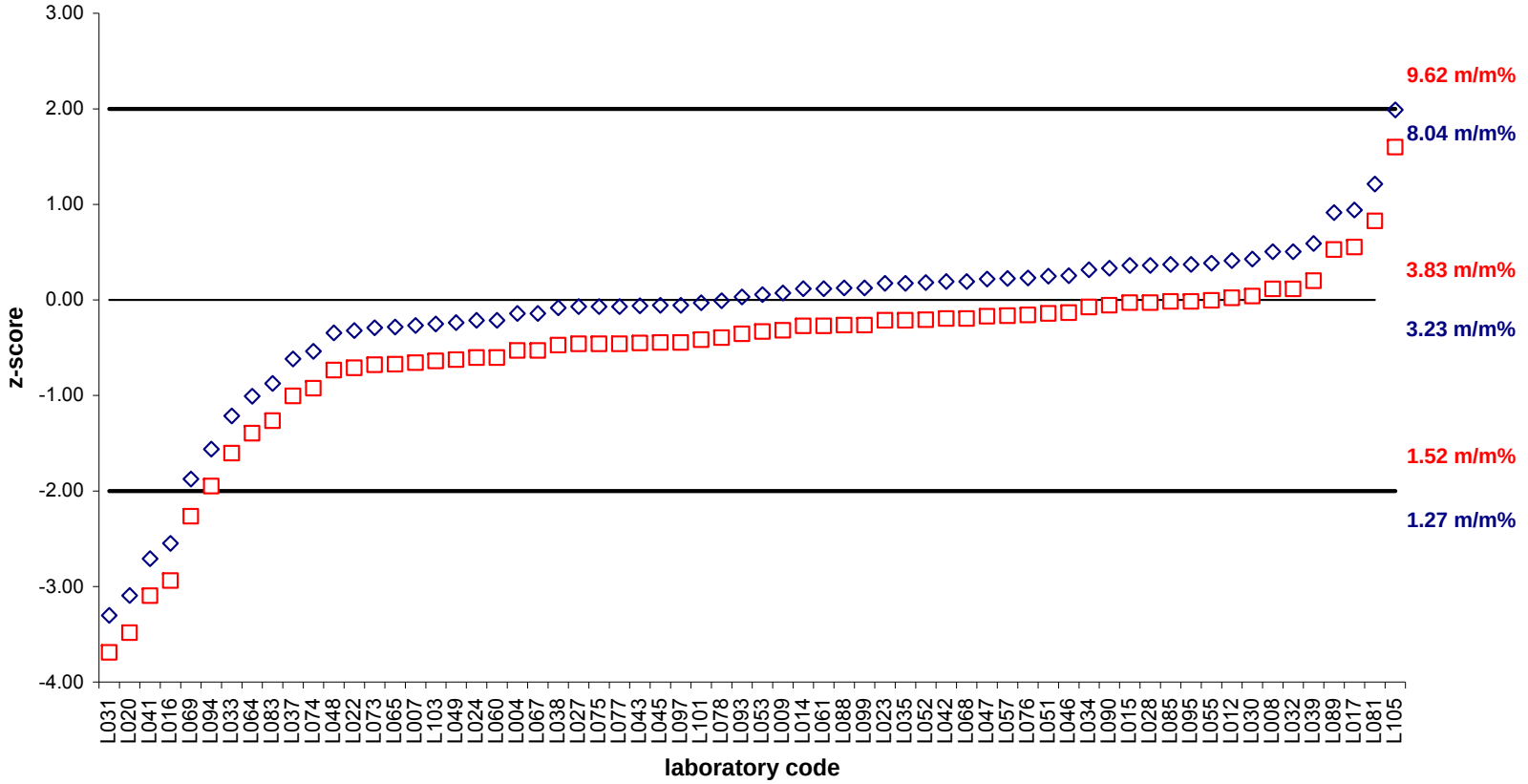


Figure 8. z-scores for maize event MON 810 maize powder level 2 on the basis of a robust mean of 2.88 cp/cp % obtained with a genomic DNA calibrant

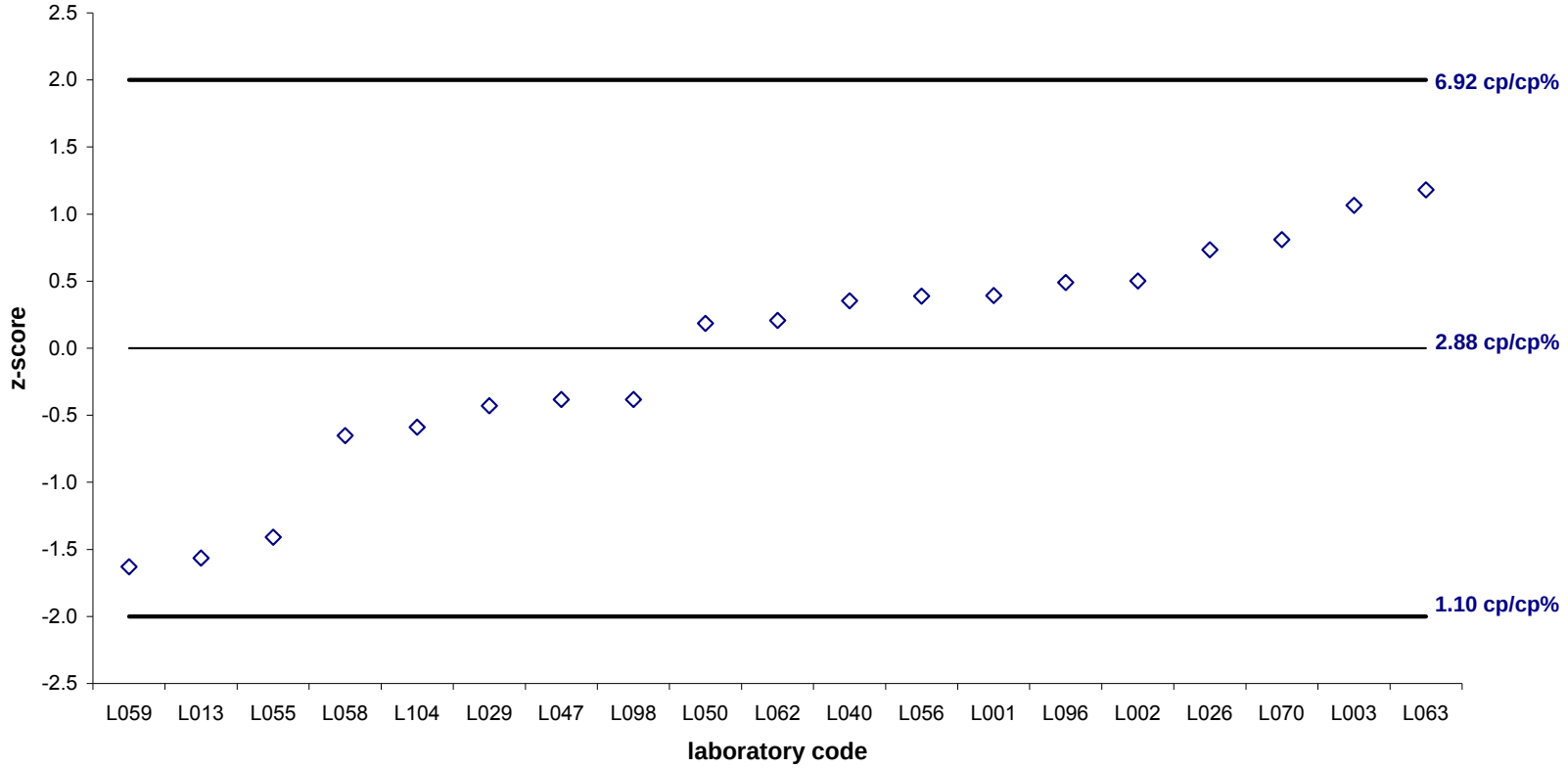
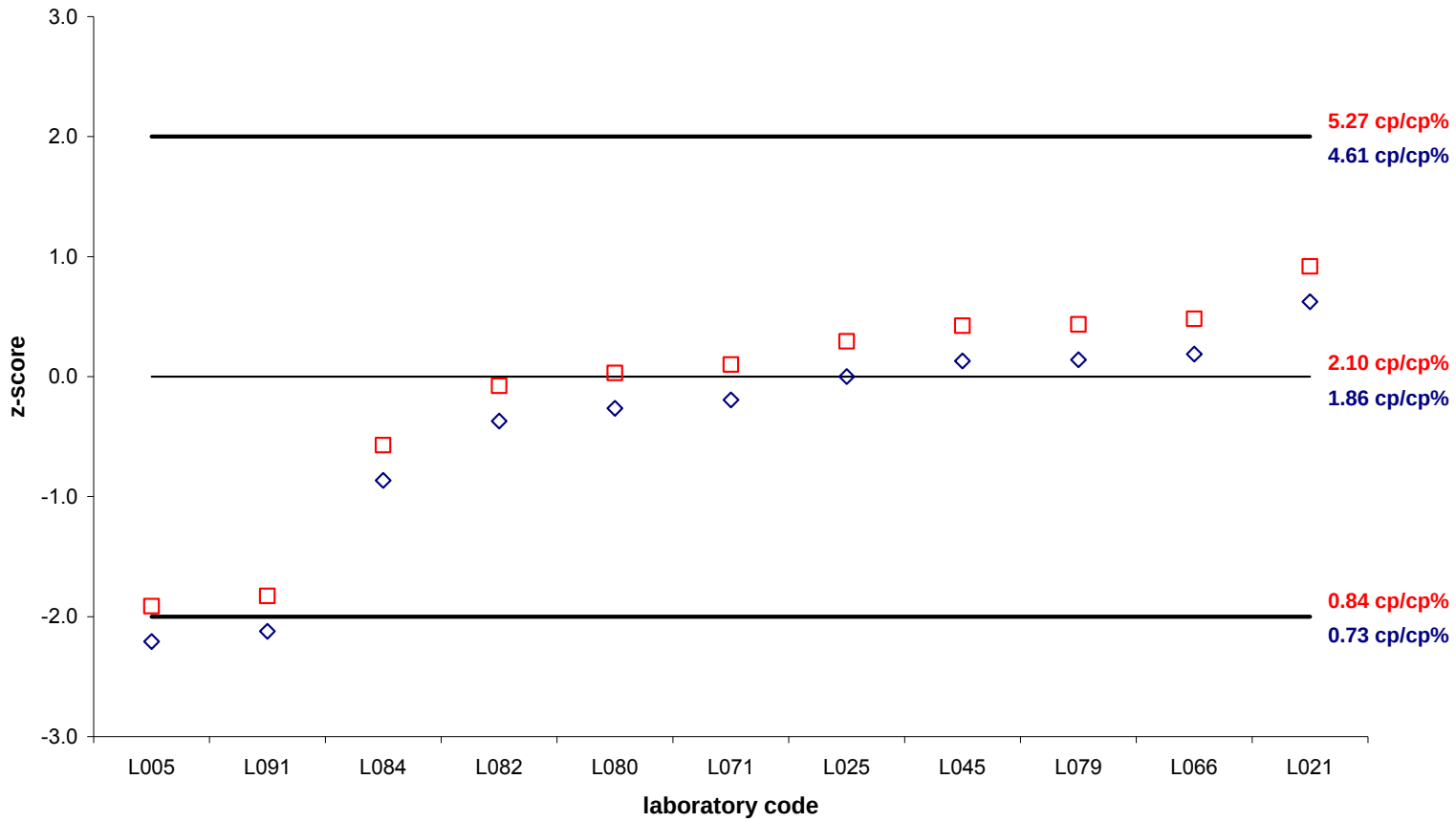


Figure 9. z-scores for maize event MON 810 powder level 2 on the basis of an assigned value of 2.10 cp/cp % (□) and a robust mean of 1.86 cp/cp % (◊) obtained with a plasmid DNA calibrant



6. Interpretation of z-scores

In general one assumes a normal distribution when calculating z-scores. In which case there is a 5 % probability that some z-scores will fall outside the working range of -2 to +2 and a 0.3 % probability that some z-scores will fall outside the working range of -3 to +3. A z-score outside the working range of -2 to +2 indicates that a participant is probably not performing according to specifications although this cannot be stated with 100 % certainty. The higher the value of the standard deviation for comparative testing $\hat{\sigma}$, the more likely participants with a z-score outside the working range of -2 to +2 are underperforming. However, a higher $\hat{\sigma}$ will also increase the probability of accepting unsatisfactory measurement results. Hence, a compromise should be made between the choice of the value of $\hat{\sigma}$ and the attempt to assess the participants' performance. In any case a z-score outside the working range of -3 to +3 will quite clearly identify an underperforming participant and will require follow-up. It should be taken into consideration that a laboratory performing well has a 5 % probability of obtaining a z-score outside the working range of -2 to +2 by mere chance.

7. Evaluation of results

The outcome of this second exercise was in general very satisfactory with a share of 82-100 % of participants exhibiting a z-score in the range of -2 to +2 for maize powder levels 1 and 2, respectively.

For the results expressed in m/m % the assigned values determined by the RM Unit of IRMM and the consensus values determined by the EURL-GMFF through robust statistics were 0.81 % versus 0.76 % for level 1, respectively and 3.83 % versus 3.23 % for level 2, respectively. Hence, the number of z-scores outside the working range of -2 to +2 was almost identical for both approaches used to determine a reference value (five z-scores for the assigned value versus four z-scores outside the working range for the robust mean for level 2). For the results expressed in cp/cp % using a plasmid DNA calibrant the assigned values determined during a previous interlaboratory comparison[13] and the consensus values determined by the EURL-GMFF through robust statistics were 0.45 % versus 0.55 % for level 1, respectively and 2.10 % versus 1.86 % for level 2, respectively. For maize powder GM level 2 the number of z-scores outside the working range of -2 to +2 was different for both approaches used to determine a reference value (two z-scores for the assigned value versus no z-scores outside the working range for the robust mean). It must be mentioned that the z-scores calculated on the basis of the assigned value were close to the limit (-1.91 for L005 and -1.83 for L091). The results expressed in cp/cp % using a genomic DNA calibrant were 20-25 % lower compared to those in m/m % (0.65 cp/cp % versus 0.81 m/m % and 2.88 cp/cp % versus 3.83 m/m % for maize powder levels 1 and 2, respectively). The robust means expressed in cp/cp % using a genomic DNA calibrant were 18-

55 % higher compared to the results expressed in cp/cp % using a plasmid DNA calibrant (0.65 cp/cp % versus 0.55 cp/cp % and 2.88 cp/cp % versus 1.86 cp/cp % for maize powder levels 1 and 2, respectively).

Nine laboratories (L005, L016, L020, L031, L041, L060, L069, L091 and L094) obtained z-scores outside the working range of -2 to +2, usually for both maize powder levels (L005, L020, L031, L041, L069 and L091) (Tables 3, 4b, 5 and 6b). L060 and L094 obtained z-scores outside the working range of -2 to +2 for maize powder level 1 whereas L016 obtained a z-score outside the working range of -2 to +2 for maize powder level 2 (Tables 3 and 5, respectively). Some laboratories (L005, L091 and L069) obtained a z-score outside the working range of -2 to +2 calculated on the basis of the assigned value but not on the basis of the robust mean (Tables 6b and 5, respectively). In the first instance, these laboratories were asked to provide their raw data so that the EURL-GMFF could investigate the cause for these z-scores. In the case of L005, L020, L031, L041 and L091 it was suspected that they had swapped the values reported for maize powder levels 1 and 2. For L016 the GM contents of maize powder levels 1 and 2 were quite close to one another, making it difficult to judge whether this laboratory had also swapped the results reported for GM levels 1 and 2. For this laboratory it was noted that the slope (-2.95) and the coefficient of determination ($R^2 = 0.977$) of the calibration curve were poor compared to the values ($-3.6 \leq$ slope ≤ -3.1, $R^2 \geq 0.98$) outlined in the ENGL guidance[20] document. The same observation was made for L060 exhibiting a slope of -2.08 and an R^2 coefficient of 0.96. In the case of L069 it was suspected that the GM content was systematically underestimated since the quality control materials included in the experiments were underestimated by a factor 2. Likewise, for L094, an underestimation of the GM content by a factor 3 was noted. The laboratory did not provide any raw data because it is no longer appointed as a NRL.

L005, L016, L020, L031, L041, L060, L069 and L091 were asked to repeat the experimental work. L094 was not asked to repeat the experimental work because it is no longer a NRL. Eight new sets of test items were shipped to these participants on the 16th of February 2011. The deadline for submission of results was the 1st of April 2011. All laboratories repeated the experimental work and submitted results within the deadline (Table 7).

Only 37 out of 90 laboratories that participated in the study provided information on measurement uncertainty (MU) of submitted results in a complete and consistent manner. This suggests that there is a need to provide participating laboratories with guidance and training on MU to harmonise the MU reported in the field of GMO detection.

8. Performance of NRLs

The second comparative testing round showed an overall positive performance of the participating NRLs.

Two NRLs (L087 and L100) registered for the second comparative testing round but did not report any results. Neither NRL gave a reason for not reporting results.

Six (L005, L031, L041, L069, L091 and L094) out of 65 NRLs, obtained z-scores outside the working range of -2 to +2. Analysing the raw data of those participants allowed identifying possible causes for these results. Five NRLs were asked to repeat the experimental work related to this second exercise. As L094 is no longer a NRL, the laboratory did not repeat the experimental work. Before the shipment of a new set of test items advice was provided regarding the approach to be followed for the experimental analyses.

In the case of L005, L031, L041 and L091 it was suspected that the participants had swapped the measurement results reported for maize powder levels 1 and 2. Those laboratories should pay particular attention to the registration and labelling of incoming samples. Obviously, such a mistake could have a major impact on routine analytical results and on the decision to label a material as above the legal threshold of 0.9 %.

As L041 showed z-scores outside the working range of -2 to +2 in two consecutive comparative testing rounds the protocol for the management of underperforming NRLs was applied. Two colleagues of the EURL-GMFF visited the laboratory from 18-20 April 2011. The outcome of the visit was very positive. Some critical issues could easily be solved through a better communication and increased networking (e.g. attending the ENGL meetings, accessing the ENGLnet website). The performance of this laboratory could thus be drastically improved. Limited investment in equipment and computer software may also improve the analytical capacity of the laboratory. A report on this visit has been sent to DG SANCO.

With the exception of L016 the laboratories that repeated the experimental work obtained good results (Table 7). Z-scores for L005, L020, L031, L041, L060, L069 and L091 were in the range of -1.08 to +0.94 which indicates a good performance. Despite z-scores in the working range of -2 to +2 an analysis of the raw data of L041 and L060 revealed the need for monitoring the performance of those laboratories. A visit to L041 showed that the laboratory needs to pay attention to DNA quantification, the preparation of the dilution series for the calibration curve, the inclusion of a sufficient number of PCR replicates in the real-time PCR experiment and to mistakes in the excel file used for the calculation of the GM content. L060 should closely monitor the performance of the endogenous target system because the reported slopes (-2.75 and -5.74) of the calibration curve were poor compared to the values ($-3.6 \leq \text{slope} \leq -3.1$) outlined in the ENGL guidance[20] document. A study of the raw data of L016 showed Ct values in the range of 22.8 to 41 for the No Template Control clearly indicating a problem with contamination. This laboratory should prepare fresh stocks of all the reagents involved in DNA extraction, DNA quantification and real-time PCR.

Table 7. Repetition of experimental work: reported results in m/m % (7a) and in cp/cp % (7b) and z-scores for event MON 810 maize powder levels 1 and 2

7a

Maize event MON 810

Laboratory number	Value	Uncertainty	z-score[1]	z-score[2]
	Assigned value = 0.81 m/m %			
	Robust mean = 0.76 m/m %			
L020	0.74	-	-0.20	-0.03
L031	1.06	0.06[a,b]	0.58	0.75
L041	0.81	22%[a,b]	0.00	0.17
L060	0.76	0.28[a]	-0.14	0.03
L069	1.01	0.66[a]	0.48	0.65
	Assigned value = 3.83 m/m %			
	Robust mean = 3.23 m/m %			
L020	3.25	-	-0.36	0.03
L031	3.05	0.04[a,b]	-0.49	-0.11
L041	2.33	22%[a,b]	-1.08	-0.69
L060	3.77	0.24[a]	-0.03	0.36
L069	3.71	2.41[a]	-0.07	0.32

7b

Maize event MON 810

Laboratory number	Value	Uncertainty	z-score[1]	z-score[2]
	Plasmid DNA calibrant			
	Assigned value = 0.45 cp/cp %			
	Robust mean = 0.49 cp/cp %			
L005	0.61	0.35[a]	0.66	0.32
L091	0.62	0.16[a]	0.70	0.36
	Genomic DNA calibrant			
	Robust mean = 0.65 cp/cp %			
	Value	**Uncertainty**	**z-score[4]**	
L016	0.19	0.05[a]	-2.64	
	Plasmid DNA calibrant			
	Assigned value = 2.10 m/m %			
	Robust mean = 1.86 m/m %			
L005	2.66	0.74[a]	0.51	0.81
L091	2.83	0.76[a]	0.65	0.94
	Genomic DNA calibrant			
	Robust mean = 2.88 cp/cp %			
	Value	**Uncertainty**	**z-score[4]**	
L016	1.07	0.25[a]	-2.05	

[a] Uncertainty reported as an absolute value, [b] Inconsistent or incomplete information, [1] z-score calculated on the basis of the assigned value,[2] z-score calculated on the basis of the robust mean, LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, Results are as submitted by participants.

9. Conclusions

In this second comparative testing round participants were asked to determine the GM content in two test items containing different GM percentages of maize event MON 810. Both test items were produced under conditions defined in a previous interlaboratory study[5].

Results could be reported in either m/m % or cp/cp %. The majority of participants submitted the results in m/m %. A minority of participants submitted the results in cp/cp % using a plasmid DNA calibrant. This allowed comparing the assigned values (μ) by the test item producer (RM Unit of IRMM) with the robust means ($\hat{\mu}$) calculated by the EURL-GMFF for both measurement units. For the results expressed in m/m % the assigned values determined by the RM Unit of IRMM and the consensus values determined by the EURL-GMFF through robust statistics were 0.81 % versus 0.76 % for level 1, respectively and 3.83 % versus 3.23 % for level 2, respectively. Hence, the number of z-scores outside the working range of -2 to +2 was almost identical for both approaches applied to determine a reference value (five z-scores for the assigned value versus four z-scores outside the working range for the robust mean). For the results expressed in cp/cp % applying a plasmid DNA calibrant the assigned values determined in the frame of a previous interlaboratory comparison[13] and the consensus values determined by the EURL-GMFF through robust statistics were 0.45 % versus 0.55 % for level 1, respectively and 2.10 % versus 1.86 % for level 2, respectively. The reported results that were swapped for maize powder levels 1 and 2 (L005 and L091) had an influence on the robust mean expressed in cp/cp %. Omitting the results reported by these laboratories would give rise to a robust mean which is identical to the assigned value for maize powder level 1 (0.45 cp/cp %) and a robust mean of 2.06 % versus 2.10 %.for maize powder level 2. It is therefore obvious that the influence of outlying values has a disproportionally higher impact on a robust mean calculated on the basis of a small number of data (N = 11 for the data expressed in cp/cp % using a plasmid DNA calibrant). A re-calculation of the robust means omitting the values that were swapped for the data reported in m/m % (L020, L031 and L041) had a small (3.29 m/m % versus 3.23 m/m % for maize powder level 2) or no influence (0.76 m/m % for maize powder level 1) on the robust means. This is logical since the number of data for the calculation of the robust mean expressed in m/m % was much larger (N = 64).

The outcome of this second comparative testing round was in general positive, with 82-100 % of participants gaining a z-score in the range of -2 to +2 for both maize powder levels 1 and 2 regardless of the calibration method, the measurement unit and the approach used for calculating the z-score. Nine laboratories obtained a z-score outside the working range of -2 to +2. The performance of these laboratories will be monitored in future comparative testing rounds. If necessary, on-site visits to those participants could be foreseen to provide assistance.

Since only about 40 % of participants provided information on MU in a complete and consistent manner, there is a need to provide laboratories with guidance and training to harmonise the MU reported in the field of GMO detection.

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11. Questionnaire data

The total number of answers in the questionnaire to each question does not always correspond to the total number of reported results. This is due to the fact that some questions were not answered by the participants.

1. DNA extraction method?	**No. of laboratories**
ISO validated	33
EURL validated	5
National reference method	3
International literature	5
In-house developed and optimised	16
Other	28

1.3. Was the DNA extraction method used within the scope of your ISO/IEC 17025 accreditation?	**No. of laboratories**
Yes	73
No	16

2. Number of replicate DNA extractions from test material?	No. of laboratories
1	1
2	62
3	13
4	12

3. Sample intake (in g) for the DNA extraction?	No. of laboratories
< 0.1	2
0.1 – 0.2	67
> 0.2	21

4. DNA extraction method/kit used?	No. of laboratories
CTAB	36
CTAB-derived	8
Dellaporta	0
Dellaporta-derived	0
Biotecon	2
DNA sorb A	0
Extragen	0
GeneScan GENE*Spin*	6
Guanidine with proteinase K	4
Macherey Nagel Nucleospin	10
Nippongene GM quicker 2	0
Promega Wizard	3
QIAmp Stool	0
Qiagen DNeasy plant mini kit	11
Qiagen QIAquick	0
R-Biopharm Rhone	0
TEPNEL kit	1
Proprietary method	0
Other	9

5. How was the clean-up of the DNA performed?	No. of laboratories
No DNA-clean-up	47
Ethanol precipitation	13
PEG precipitation	0
Amersham MicroSpin S300	0
Promega Wizard DNA-clean-up resin	9

Qiagen QIAquick	5
Qiagen Genomic-Tip 20/G	1
Silica	7
Propietary method	0
Other	8

6. How have you quantified the DNA?	**No. of laboratories**
Gel	2
UV spectrophotometer	37
Nanodrop	27
Fluorometer	15
Other	4
DNA was not quantified	5

7. What was the DNA concentration (in ng/µL) of the undiluted extracted sample?	**No. of laboratories**
0-50	10
50-100	18
100-150	13
150-200	19
200-250	5
250-300	5
300-350	2
350-400	4
400-450	1
450-500	2
500-550	1
550-600	3
600-650	0
650-700	2
700-750	0
750-800	0
800-850	0
850-900	0
900-950	1
950-1000	0
Not applicable	3

8. Dilution buffer?	**No. of laboratories**
TE (10 mM Tris-HCl, 1 mM EDTA)	14
TE 0.1X (10 mM Tris-HCl, 0.1 mM EDTA)	11
TE low (1 mM Tris-HCl, 0.01 mM EDTA)	3

Water	50
Other	12

9. Validation status of the PCR analytical method?	**No. of laboratories**
ISO validated	21
EURL validated	42
National reference method	1
International literature	4
In-house developed and optimised	9
Other	13

9.3. Was the PCR analytical method used within the scope of your ISO/IEC 17025 accreditation?	**No. of laboratories**
Yes	63
No	26

10. Real-time PCR analytical method	**No. of laboratories**
Multiplex PCR	4
Singleplex PCR	86

11. Real-time PCR instrument?	**No. of laboratories**
ABI 7000	4
ABI 7300	6
ABI 7500	25
ABI 7700	4
ABI 7900HT	25
ABI StepOne & StepOnePlus real-time PCR system	1
BioRad icycler	4
Corbett Rotor-Gene 6000	1
Realplex	0
Roche LightCycler 2.0	4
Roche Lightcycler 480	4
Stratagene Mx3000/Mx3005	4
Stratagene Mx4000	0
Other	8

12. Real-time PCR plate	**No. of laboratories**
96-well plate	79

384-well plate	1
other	10

13. Real-time PCR mastermix	**No. of laboratories**
ABI TaqMan® Universal PCR master mix	41
ABI TaqMan® Universal PCR master mix, no AmpErase® UNG	5
ABI TaqMan® Fast Universal PCR master mix	0
ABI TaqMan® PCR Core Reagent Mix	9
ABI TaqMan® Gold with Buffer A	1
Agilent Technologies: Brilliant® II SYBR® Green QPCR Master Mix	0
Agilent Technologies: Brilliant® QPCR Master Mix	0
Bio-Rad: iTaq Fast Supermix With ROX	1
Bio-Rad: iQ SYBR Green Supermix	0
Eurogentec: FAST qPCR MasterMix for SYBR® Green I	0
Eurogentec: FAST qPCR MasterMix Plus	0
Promega GoTaq® qPCR master mix	0
Sigma JumpstartTM Taq ReadyMix TM	1
Proprietary real-time PCR master mix	0
Other reaction mixes	33 of which :
No information given	1
ABI AmpliTaq Gold DNA Polymerase with GeneAmp 10X PCR Buffer	1
ABI TaqMan Environmental master mix v2.0	1
ABI TaqMan GMO Maize PCR Mix	1
Congen SureFood GMO MON810 Corn-Kit	2
Diagenode 2x mastermix	3
Eurofins reaction mix event MON810,	6
Eurogentec qPCR MasterMix Plus	1
Eurogentec qPCR Mastermix Plus without UNG for probe	2
Eurogentec qPCR Core Kit - No ROX	1
Fermentas Maxima Probe/ROX qPCR Master Mix	1
Home made	1
Metabion mi-Taq polymerase + dNTPs	1
QIAGEN QuanitiTect Probe PCR Kit	2
Qiagen QuantiTect Probe RT-PCR master mix	1
Roche LightCycler FastStart DNA Master	2
Roche LightCycler 480 Probes Master	3
Roche LightCycler TaqMan Master	1
R-biopharm master mix	1
Thermo Fisher Scientific Absolute Fast Q-PCR low	1

ROX-Mix

13.2. Number of reagents involved	No. of laboratories
1	2
2	2
3	5
4	14
5	34
6	9
> 6	24

14.1. Sample intake (in ng) per real-time PCR reaction	No. of laboratories
0-100	45
100-200	34
200-300	4
300-400	3
400-500	3
> 500	1

Questions 14.2 to 14.5 only had to be answered in case of different sample intakes per real-time PCR

14.2. Sample intake (in ng) per real-time PCR reaction	No. of laboratories
0-100	14
100-200	13
200-300	1
300-400	1
400-500	2
> 500	1

14.3. Sample intake (in ng) per real-time PCR reaction	No. of laboratories
0-100	10
100-200	6
200-300	0
300-400	1
400-500	2
> 500	0

14.4. Sample intake (in ng) per real-time	No. of laboratories

PCR reaction	
0-100	8
100-200	5
200-300	0
300-400	0
400-500	3
> 500	0

14.5. Sample intake (in ng) per real-time PCR reaction	No. of laboratories
0-100	4
100-200	4
200-300	0
300-400	0
400-500	1
> 500	0

15.1. Sample intake (in µL) per real-time PCR reaction	No. of laboratories
1	2
2	6
3	5
4	4
5	63
6-10	5
> 10	4

16. Number of reactions per DNA extraction	No. of laboratories
1	0
2	31
3	29
4	12
5	3
6	9
> 6	5

17. Number of real-time PCR cycles	No. of laboratories
40	10
42	1
45	68
47	0

50	7
Other	4

18. Real-time PCR detection method used?	**No. of laboratories**
MGB	0
Roche probe	0
Taqman probe	89
SYBRGreen	0
Other	1

19. Real-time PCR quantification method used?	**No. of laboratories**
DNA copy number standard curve using a dilution series	37
Mass/mass standard curve using a dilution series	37
Delta Ct method	15
Other of which :	1
Digital PCR	1

20. For standard curve approach: slope - endogenous gene	**No. of laboratories**
Within Minimum Performance Requirements (MPR)[20]: -3.6 ≤ slope ≤ -3.1	63
Outside MPR	26

21. For standard curve approach: slope – GM trait gene	**No. of laboratories**
Within MPR: -3.6 ≤ slope ≤ -3.1	58
Outside MPR	32

22. For standard curve approach: R^2 coefficient - endogenous gene	**No. of laboratories**
≥ 0.98	71
Outside MPR	17

23. For standard curve approach: R^2 coefficient – GM trait gene	**No. of laboratories**
≥ 0.98	73
Outside MPR	16

24. For standard curve approach: dynamic working range of the calibration curve - endogenous gene	No. of laboratories
$1.00x10^5$ - $1.00x10^2$ cp	5
$1.10x10^5$ - $1.80x10^4$ cp	1
$2.05x10^5$ - $2.00x10^2$ cp	1
$2.88x10^4$ - $1.80x10^3$ cp	1
$2.89x10^4$ - $3.61x10^2$ cp	1
$3.67x10^4$ - $1.84x10^3$ cp	1
$4.00x10^4$ - $2.00x10^2$ cp	1
$4.81x10^4$ - $7.52x10^3$ cp	1
$5.00x10^4$ - $1.00x10^2$ cp	1
$5.00x10^4$ - $4.00x10^2$ cp	1
$5.00x10^5$ - $1.00x10^3$ cp	1
$5.00x10^5$ - $5.00x10^1$ cp	1
$5.00x10^6$ - $5.00x10^1$ cp	1
$5.40x10^4$ - $8.60x10^1$ cp	1
$5.50x10^4$ - $2.15x10^2$ cp	1
$5.50x10^4$ - $3.82x10^2$ cp	1
$5.50x10^5$ - $1.00x10^3$ cp	1
$6.05x10^4$ - $2.36x10^2$ cp	1
$6.25x10^4$ - $4.00x10^1$ cp	1
$7.34x10^4$ - $5.10x10^2$ cp	1
$7.34x10^4$ - $5.87x10^2$ cp	1
$7.87x10^4$ - $2.00x10^1$ cp	1
$8.19x10^4$ - $1.60x10^2$ cp	6
$8.26x10^4$ - $7.65x10^2$ cp	1
$9.07x10^4$ - $1.48x10^2$ cp	1
$9.47x10^4$ - $1.97x10^2$ cp	1
100 - 0.16 ng	1
125 - 3.9 ng	1
200- 0.32 ng	2
300 - 1 ng	1
500 - 1 ng	1
100 - 1.20 %	1
100 % Reference Material	1
5 - 0.01 %	1
5 - 0.31 %	1
Inconsistent reporting	14

25. For standard curve	No. of laboratories

approach: dynamic working range of the calibration curve - GM trait gene	
$1.00x10^4$ - $2.50x10^1$ cp	1
$1.00x10^4$ - $5.00x10^1$ cp	2
$1.02x10^4$ - $4.00x10^1$ cp	7
$1.35x10^3$ - 5 cp	1
$1.41x10^4$ - $3.80x10^1$ cp	1
$1.44x10^3$ - $9.00x10^1$ cp	1
$1.45x10^3$ - $1.80x10^1$ cp	1
$1.84x10^3$ - $2.50x10^1$ cp	1
$1.84x10^3$ - $9.20x10^1$ cp	1
$2.00x10^3$ - $1.00x10^1$ cp	1
$2.01x10^3$ - $2.00x10^1$ cp	1
$2.37x10^3$ - $4.90x10^1$ cp	1
$2.41x10^3$ - $3.80x10^1$ cp	1
$2.41x10^3$ - $3.80x10^1$ cp	1
$2.50x10^3$ - $5.00x10^1$ cp	1
$2.75x10^3$ - $1.10x10^1$ cp	1
$2.75x10^3$ - $1.90x10^1$ cp	1
$3.03x10^3$ - $1.20x10^1$ cp	1
$3.12x10^3$ - $2.00x10^1$ cp	1
$3.30x10^3$ - $3.10x10^1$ cp	1
$3.67x10^3$ - $2.90x10^1$ cp	1
$3.67x10^3$ - $4.00x10^1$ cp	1
$4.40x10^3$ - $7.10x10^1$ cp	1
$5.00x10^4$ - $2.50x10^1$ cp	2
$5.00x10^5$ - $5.00x10^1$ cp	1
$5.00x10^6$ - $5.00x10^1$ cp	1
$6.25x10^4$ - $4.00x10^1$ cp	1
10 - 0.016 ng	1
125 - 3.9 ng	1
25 - 0.05 ng	1
40 - 0.016 ng	1
5.0 - 0.008 ng	1
15 - 0.05 %	1
5.0 - 0.01 %	1
5.0 - 0.06 %	1
5.0 - 0.31 %	1
Inconsistent reporting	15

26. For Delta Ct method: slope	**No. of laboratories**
Within MPR: -3.6 ≤ slope ≤ -3.1	18

Outside MPR	6

27. For Delta Ct method: R^2 coefficient	**No. of laboratories**
≥ 0.98	23
Outside MPR	3

28. For Delta Ct method: dynamic working range of the calibration curve	**No. of laboratories**
5 - < 0.02 %	1
5 - 0.1 %	3
Inconsistent reporting	13

29. Endogenous target DNA sequences for MON810 maize?	**No. of laboratories**
Adh	7
Hmg	62
Invertase	3
Zein	5
zSSIIb	11
Other	2

30. Amplicon size (in bp) – endogenous gene	**No. of laboratories**
68	2
79	59
82	6
92	2
100	1
121	1
127	1
134	3
135	1
136	1
151	10
277	1

31. Primer and probe sequences – endogenous gene	
31.1 F-primer	**No. Of laboratories**
CCAATCCTTTGACATCTGCTCC	1
CGTCGTTTCCCATCTCTTCCTCC	4

CGTCGTTTCCCATCTCTTCCTCCT	1
CTCCCAATCCTTTGACATCTG	1
CTCCCAATCCTTTGACATCTGC	9
GTACCGGAACTACAAGGAGA	1
TCGAAGGACGAAGGACTCTAACGT	1
TGCAGCAACTGTTGGCCTTAC	2
TGGCGGACGACGACTTGT	3
TTGGACTAGAAATCTCGTGCTA	1
TTGGACTAGAAATCTCGTGCTGA	53
Other, unknown or not provided	11

31.2 R-primer	**No. Of laboratories**
AAAGTTTGGAGGCTGCCGT	3
CCACTCCGAGACCCTCAGTC	5
GAGCACGTCCTCATACAGCA	1
GATCAGCTTTGGGTCCGGA	1
GCCACCTTCCTTTTCCACTATCTT	1
GCTACATAGGGAGCCTTGTCC	1
GCTACATAGGGAGCCTTGTCCT	53
TCGATTTCTCTCTTGGTGACAGG	13
Other, unknown or not provided	11

31.3 Probe	**No. Of laboratories**
AACATCCTTTGCCATTGCCCAGC	1
AATCAGGGCTCATTTTCTCGCTCCTCA	5
AGCAAAGTCAGAGAGCTGCAATGCA	10
ATCATCACTGGCATCGTCTGAAGCGG	2
CAATCCACACAAACGCACGCGTA	54
CGAGCAGACCGCCGTGTACTTCTACC	3
CGGCATGGCGCAGGACCTCA	1
GCAAAGTCAGAGCGCTGCAATGCA	1
Other, unknown or not provided	12

32. GM trait target DNA sequence for MON 810 maize?	**No. of laboratories**
35S promoter	5
CryIAb	2
MON 810-specific	80
Nos terminator	0
Other, , unknown or not provided	3

33. Amplicon size (in bp) – GM trait gene	**No. of laboratories**
79	1

92	66
103	1
106	1
113	10
115	5
123	1
143	1
154	1
Other, unknown or not provided	2

34. Primer and probe sequences – GM trait gene

34.1. F-primer	**No. Of laboratories**
CAAGTGTGCCCACCACAGC	1
CCACCACAGCCACCACTTCT	1
CCTTCATAACCTTCGCCCG	6
GATGCCTTCTCCCTAGTGTTGA	9
GTAGCCTTCTCCCTAGTGTTGA	1
TCGAAGGACGAAGGACTCTAACGT	58
TTGGACTAGAAATCTCGTGCTGA	1
Other, unknown or not provided	13

34.2. R-primer	**No. Of laboratories**
AATAAAGTGACAGATAGCTGGGCA	6
CTGCTCGCAAGCAAATTCGG	1
GCAAGCAAATTCGGAAATGAA	1
GCCACCTTCCTTTTCCACTATCTT	57
GCCACCTTCCTTTTCCACTATCTTTAACGT	1
GCTACATAGGGAGCCTTGTCCT	1
GGATGCACTCGTTGATGTTTG	10
Other, unknown or not provided	13

34.3. Probe	**No. Of laboratories**
AACATCCTTTGCCATTCGCCAGC	1
AACATCCTTTGCCATTGCCATTGCCCAGC	2
AACATCCTTTGCCATTGCCCA	1
AACATCCTTTGCCATTGCCCAGC	52
AACATCCTTTGCCATTGCCCAGCT	1
AACATCCTTTGCCATTGCCCATC	1
ACCGACCTGAACGAGGACTT	1
ACGAAGGACTCTAACGTTTAACATCCTTTGCCA	5
ACGAAGGACTCTAACGTTTAACATCTTTTGCCA	1
AGATACCAAGCGGCCATGGACAACAA	10
CAATCCACACAAACGCACGCGTA	1
CGACCTGAACGAGGACTTTCGGTAGCC	1
Other, unknown or not provided	13

35. Which reference material was used for calibration?	No. of laboratories
Congen SureFood GMO MON 810 Corn-Kit	2
ERM-AD413	5
ERM-AD413 + ERM-BF413 series	1
ERM-AD413 + ERM-BF413f	1
ERM-BF413 (not specified)	9
ERM-BF413 series	9
ERM-BF413a	1
ERM-BF413b+c+e+f	2
ERM-BF413d	3
ERM-BF413e	1
ERM-BF413f	45
ERM-BF413f + ERM-BF412f	1
GEMM15A	1
Not applicable	2
Plasmid DNA Eurofins	5
Plasmid Nippon Gene	1

36. Which reference material was used for quality control?	No. of laboratories
ERM-AD413 + ERM-BF413 series	1
ERM-BF410gk	1
ERM-BF413 (not specified)	7
ERM-BF413 (not specified) + in-house control	1
ERM-BF413 series	3
ERM-BF413a	3
ERM-BF413a+b+c+d+e	1
ERM-BF413a+b+d	2
ERM-BF413a+b+d+e	1
ERM-BF413a+c	1
ERM-BF413a+d	1
ERM-BF413b	1
ERM-BF413b+c+d+e+f	1
ERM-BF413b+c+e+f	1
ERM-BF413b+d	7
ERM-BF413b+d+e	1
ERM-BF413b+d+f	2
ERM-BF413b+f	1
ERM-BF413c	5
ERM-BF413c+d+e	1
ERM-BF-413c+e	1

ERM-BF413d	31
ERM-BF413d+f	1
ERM-BF413e	4
ERM-BF413f	5
GEMM15A	1
None	1
Water	1

37. Did you report uncertainty as an absolute value?	**No. of laboratories**
Yes	48
No	42

37.1. If you have responded yes to 37, does the uncertainty correspond to a repeatability standard deviation?	**No. of laboratories**
Yes	26
No	17
Not applicable	18

37.2. If you have responded no to 37.1, does the uncertainty correspond to a within-laboratory reproducibility?	**No. of laboratories**
Yes	20
No	13
Not applicable	21

37.3. Does the uncertainty include a contribution from the heterogeneity of the material?	**No. of laboratories**
Yes	8
No	33
Not applicable	19

37.6. Did you report an expanded uncertainty including a coverage factor?	**No. of laboratories**
Yes	40
No	19
Not applicable	12

37.7. If you have responded yes to 37.6, please specify the coverage factor used (k=1	**No. of laboratories**

for a 66.67 % confidence level, k = 2 for a 95 % confidence level, k = 3 for a 99 % confidence level).	
1	1
2	36
3	1
other	2

38. Did you report the uncertainty as a relative value (i.e. in %)?	**No. of laboratories**
Yes	34
No	41

38.1. If you have responded yes to 38, does the value reported correspond to a %age of the GM level reported?	**No. of laboratories**
Yes	20
No	12
Not applicable	15

38.2. Does the uncertainty correspond to a relative repeatability standard deviation?	**No. of laboratories**
Yes	18
No	11
Not applicable	18

38.3. If you have responded no to 38.2, does the uncertainty correspond to a relative within-laboratory reproducibility?	**No. of laboratories**
Yes	10
No	7
Not applicable	20

38.4. Does the uncertainty include a contribution from the heterogeneity of the material?	**No. of laboratories**
Yes	11
No	32

38.7. Did you report an expanded uncertainty including a coverage factor?	**No. of laboratories**
Yes	23
No	17

Not applicable	10

38.8. If you have responded yes to 38.7, please specify the coverage factor used (k=1 for a 66.67 % confidence level, k = 2 for a 95 % confidence level, k = 3 for a 99 % confidence level).	**No. of laboratories**
1	0
2	23
3	0
other	0

Practical LOD (in %) of the GM content determination in mass/mass for level 1	**No. of laboratories**
< 0.01	5
0.01	20
0.02	10
0.03	7
0.04	2
0.05	12
0.06	2
0.07	2
0.08	2
0.09	0
0.1	12
> 0.1	5

Practical LOQ (in %) of the GM content determination in mass/mass for level 2	**No. of laboratories**
< 0.01	0
0.01	1
0.02	1
0.03	2
0.04	3
0.05	1
0.06	2
0.07	2
0.08	1
0.09	3
0.1	52
> 0.1	13

12. Acknowledgements

We sincerely thank Roberta Brustio, Maddalena Chessa, Stéphane Cordeil, Barbara Munaro, Steven Price, Eleonora Scigliano, Pierluigi Tenuta, Stefania Tomasina of the MBG Unit and EURL-GMFF for their invaluable contributions to this second comparative testing round. A special thanks to Marko Maras who is very actively involved in the comparative testing activities. We are grateful to Philippe Corbisier, Hendrik Emons, Brigitte Fontenelle, Anne-Marie Kortekaas, Stefanie Trapmann, Ingrid Wuyts from the RM Unit of IRMM for the production of the test items and for taking care of the shipment of test items. We acknowledge Fernando Cordeiro Raposo, Beatriz De la Calle, Franz Ulberth, Inge Verbist from the FSQ Unit of IRMM for the on-line registration of participants and the management of the reported results.

The labs listed below are kindly acknowledged for their participation in this exercise.

Organisation	Department	Country	Status
A BioTech Lab	Laboratory for Biotechnology	RS	4
Austrian Agency for Health and Food Safety (AGES)	Competence Centre Biochemistry	AT	1, 2
Agricultural Institute of Slovenia		SI	2
Agri-Food and Veterinary Authority of Singapore	Veterinary Public Health Center	SG	4
Agroscope Liebefeld-Posieux	Analytics	CH	4
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit		DE	2
BIOMI LTD		HU	3
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit		DE	1
Central Agricultural Office, Food and Feed Safety Directorate (CAO FFSD)	Laboratory for GMO Food	HU	1, 2
Central Agricultural Office, Food and Feed Safety Directorate (CAO FFSD)	Feed Investigation NRL	HU	1, 2
Center for Agricultural Technology Augustenberg	Referat 24	DE	2
Central Control and Testing Institute of Agriculture	Dptm. of Molecular Biology	SK	1, 2
Centro Nacional de Alimentación (Agencia Española de seguridad alimentaria y nutricion)	Biotechnology Unit	ES	1, 2
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL)		DE	3
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)		DE	2
Consorcio CSIC-IRTA-UAB	SABQ	ES	3
CRA-W (Centre wallon de Recherches agronomiques)	Valorization of Agric. Prod.	BE	1, 2
Croatian National Institute of Public Health	GMO Qant. and RA Unit	HR	4
Crop Research Institute	Reference Laboratory for GMO	CZ	1, 2
CVUA Freiburg	Gentechnik	DE	2
Czech Agriculture and Food Inspection Authority	Dep. of Test.Lab.of Brno Insp.	CZ	2
Department of Chemistry		MY	4
DTU-Food, National Food Institute	Toxicology and Risk Assessment	DK	1, 2
EC-JRC-IRMM	RM	BE	3
Ente Nazionale Sementi Elette	Laboratorio Analisi Sementi	IT	2
Federal Institute for Risk Assessment (BfR)		DE	2
Federal Office of Public Health FOPH	Food Safety	CH	3
Fera*	Crop and Food Security	IE	1

Fera	Crop and Food Security	UK	2
Finnish Customs Laboratory	Et2 / BIO	FI	1, 2
Groupe d'Etude et de contrôle des Variétés et des Semences (GEVES)	BioGEVES	FR	1, 2
Hessisches Landeslabor		DE	2
Inst. Nacional de Recursos Biológicos	Unid. de Invest. de Prot. Plan	PT	2
Institut für Gesundheit und Umwelt	Gentechnik	DE	2
Institute for Agricultural and Fisheries Research	Unit Technology and Food	BE	1, 2
Institute for Diagnosis and Animal Health	Molecular Biology and GMO Unit	RO	1
Institute for genetic engineering and biotechnology		BA	4
Institute for Seed and Seedlings	Seed testing Laboratory	HR	4
Institute of Biochemistry and Biophysics		PL	2
Institute of Chemical Technology in Prague	Biochemistry and Microbiology	CZ	2
Institute of Food Safety, Animal Health and Environment „BIOR"	Virology department	LV	1, 2
Instytut Zootechniki PIB Krajowe Laboratorium Pasz	Pracownia w Szczecinie	PL	1, 2
Istituto Superiore di Sanità - National Institute of Health	DSPVSA-GMO and Mycotoxins Unit	IT	2
Istituto Zooprofilattico Sperimentale Lazio e Toscana	Biotechnology	IT	1, 2
Kyung Hee University	Food Science	KR	4
Laboratoire National de la Protection des Végétaux	OGM	FR	1, 2
Laboratoire national de santé	food control	LU	1, 2
Laboratorio Arbitral Agroalimentario - MARM	OGM	ES	1, 2
Landesamt für Umweltschutz Sachsen-Anhalt	FG13	DE	2
Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	DE	2
Landeslabor Berlin Brandenburg	Fachbereich I-6	DE	2
Landeslabor Schleswig-Holstein		DE	2
Landesuntersuchungsamt Rheinland-Pfalz	Institut f. Lebensmittelchemie	DE	2
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen (LUA)	Amtliche Lebensmitteluntersuch	DE	2
Lower Saxony Federal State Office for Consumer Protection and Food Safety (LAVES) - Lebensmittelinstitut Braunschweig	Molekularbiologie	DE	2
LGC Limited	Molecular and Cell Biology	UK	1, 2
LSGV Saarbrücken	F5 Molekularbiologie	DE	2
Ministério da Agricultura, Pecuária e Abastecimento	LANAGRO-MG	BR	4
Ministry of Agriculture and Rural affairs Provincial Control laboratory	GMO	TR	4
Ministry of Finance - General Secretariat for Tax and Custom Issues, General Chemical State Laboratory	Food Division - Laboratory	GR	1, 2
National Bureau of Plant Genetic Resources, New Delhi	NRC on DNA Fingerprinting	IN	4
National Center of Public Health Protection	Lab for GM Food analysis	BG	1, 2
National Food Administration	Research & Development	SE	1, 2
National Food and Veterinary Risk Assessment Institute	Molecular biology and GMO section	LT	1, 2
National Food Reference Laboratory	Biotechnology and GMO Lab.	TR	4
National Institute for Food and Drug Surveillance - INVIMA	GMO Detection Laboratory	CO	4
National Institute of Biology		SI	1, 2
National Institute of Public Health	Department for Food Safety and Nutrition	CZ	2

National Quality Control Laboratory of Drug and Food	Biotechnology Laboratory	ID	4
National Research and Development Institute for Biotechnology in Horticulture	Research	RO	4
National Veterinary Research Institute	Hygiene of Animal Feedingstuff	PL	1, 2
Plant Breeding and Acclimatization Institute - National Research Institute	GMO Controlling Laboratory	PL	2
Quality Assurance and Testing Center 3	Microbiology-GMO testing lab	VN	4
RIKILT -Institute of Food Safety, WUR	NFA	NL	1, 2
Science and Advice for Scottish Agriculture (SASA)	Diagnostics & Mol. Biology	UK	2
Scientific Institute of Public Health	Platform Biotech & Mol Biol	BE	1, 2
Service Commun des Laboratoires du MINEFI - Laboratoire de Strasbourg		FR	1, 2
Servicio Agricola y Ganadero	De laboratorios y estaciones c	CL	4
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Geschäftsbereich 6	DE	2
State Office for Agriculture, Food Safety and Fishery Mecklenburg-Western Pomerania	Molecular Diagnostics	DE	2
State Veterinary and Food Institute Dolny Kubin	Dept. of mol. biol. analysis	SK	1, 2
Tallinn University of Technology	Gene Technology	EE	2
The Danish Plant Pirectorate	Diagnose lab.	DK	1, 2
Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz	Lab for detection of GMO in Food	DE	2
Thüringer Landesanstalt für Landwirtschaft	Abteilung Untersuchungswesen	DE	3
Ukrmetrteststandard		UA	4
Umweltbundesamt	Landuse & Biosafety	AT	1, 2
US Department of Agriculture	Biotechnology	US	4
Veterinary and Food Laboratory		EE	1, 2
The Food and Consumer Product Safety Authority	Laboratory	NL	2

1 Laboratory appointed under Regulation (EC) No 882/2004, 2 Laboratory appointed under Regulation (EC) No 1981/2006, 3 ENGL only member, 4 Laboratory from third country, *Fera also participated as NRL for Ireland

13. Annex 1: Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 22 June 2010
JRC/04/MBG/GVDF/st/Ares(2010)355932

To: **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.**

All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the European Union reference laboratory for genetically modified organisms

Re: **Invitation to participate in the comparative test ILC-CRL-GMFF-CT-02/10**

Under Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) shall organise comparative testing and ensure an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols. Hereby, I would like to invite you to participate in the second round of comparative testing ILC-CRL-GMFF-CT-02/10. This round of comparative testing will include two test materials of maize MON 810. The participant will need to quantify the GM level in each test material.

I would like to remind you that participation in comparative testing is mandatory for all NRLs nominated under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006. Your participation is free of charge.

Comparative testing is organised by the EURL-GMFF in collaboration with the Institute for Reference Materials and Measurements (IRMM, Geel, BE). Registration for the second round of comparative testing and submission of results will be handled by IRMM. Please register electronically for the second comparative testing round using the following link: https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=520

Please be aware that you need to submit multiple registration forms when you wish to apply different approaches of quantification (i.e. standard curve method, delta Ct method,…) or use different units of measurement for reporting your results.
Once you have submitted your registration electronically, print your registration form, sign it and send it to IRMM by fax or E-mail:

Fax: 132 14 571 865
Mail: JRC-IRMM-IMEP@ec.europa.eu
Cc to: mbg-comparative-testing@jrc.ec.europa.eu

Joint Research Centre I-21027 Ispra (VA), Italy
Telephone: direct line (+39-0332) 78 5239· Telefax: (+39-0332) 78 5483.
E mail: guy.van-den-eede@ec.europa.eu
http://mbg.jrc.ec.europa.eu

Your fax/E-mail is the confirmation of your participation.

The deadline for registration is **1 July 2010**. Samples should be shipped during the week of **6 to 10 September 2010**. The deadline for submission of results is **22 October 2010**.

If you should have any questions related to the second round of comparative testing, please contact:

Diana Charels
European Commission – Joint Research Centre
Molecular Biology and Genomics Unit – TP331
Via E. Fermi 2749
I-21027 Ispra (VA)
Phone: +39 0332 78 6518
Fax: +39 0332 78 6322
E-mail: mbg-comparative-testing@jrc.ec.europa.eu

The EURL-GMFF is looking forward to your participation.

Yours sincerely,

Guy Van den Eede
Head of Molecular Biology and Genomics Unit

Joint Research Centre I-21027 Ispra (VA), Italy
Telephone: direct line (+39-0332) 78 5239- Telefax: (+39-0332) 78 5483.
E-mail: guy.van-den-eede@ec.europa.eu
http://ihcp.jrc.ec.europa.eu

14. Annex 2: Accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Ispra, 31 August 2010
JRCI04/MBG/GVDE/mc/ Ares(2010)548664

«Address»

Subject: Participation in ILC-CRL-GMFF-CT-02/10, a comparative testing r ound to quantify the GM content of maize MON 810 test items.

Dear «Name» «Surname»,

Thank you for participating in the ILC-CRL-GMFF-CT-02/10 comparative testing round to quantify the GM content of maize MON 810 test items.

You will receive the test items shipped at room temperature via courier. The shipment will be carried out in the week of **6 to 10 September 2010**. On the day of the shipment we will inform you, by E-mail, about the parcel tracking number. Please make sure that someone in your laboratory is available to receive the parcel.

The parcel contains:

1. Two brown glass bottles each containing approximately 1 g of test item
2. An "Acknowledgement of Reception" form
3. This accompanying letter

Please check whether the glass bottles containing the test item remained undamaged during transport and return the "Acknowledgement of Reception" form by fax (+39 0332 789333). You should store the samples in a dark and cold place (not exceeding 18 ºC).

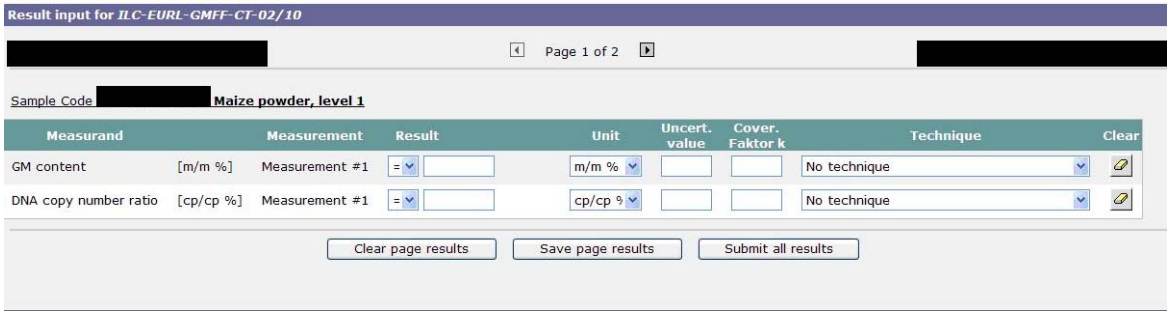
You should determine the GM level of MON 810 in each test item received. The procedure used for quantification should resemble as closely as possible the one that you use in routine sample analyses.

The results can be reported in mass/mass % and/or copy/copy % as outlined below:

$$\text{mass/mass \%} = \frac{\text{mass GM [g]}}{\text{Total mass [g]}} \times 100\ \%$$

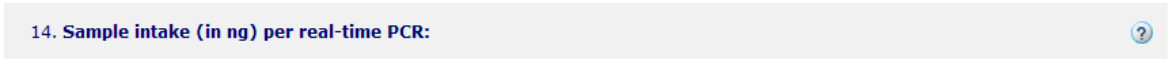
$$\text{copy/copy \%} = \frac{\text{GM DNA copy numbers [cp]}}{\text{Target taxon-specific DNA copy numbers [cp]}} \times 100\ \%$$

You can find the reporting website at https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do To access this webpage you need a personal password which is «PARTKEY». The system will guide you through the reporting procedure. Please enter for each test item the measurement result with its associated uncertainty. For maize powder level 1 the results will have to be reported on page 1 of 2 of the on-line reporting system. Please report your results either in GM content or DNA copy number ratio.



For maize powder level 2 the results will have to be reported on page 2 of 2 of the on-line reporting system.

After entering all results, please complete the questionnaire. Items bearing a question mark icon on the right-hand side, as shown in the example below, contain additional information for the participant. In the reporting website clicking on the icon will give access to this information. Do not forget to save, submit and confirm when required to do so.



The pdf file of the questionnaire that you will or have already received by E-mail is intended as an aid in the laboratory. In this pdf file, items with the word '(number)' indicate that a numerical value should be provided. Pdf files of questionnaires bearing hand-written answers will not be accepted for reporting.
Only results and answers to the questionnaire reported on-line on the reporting website https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do **will be accepted.**
Directly after submitting your results and the questionnaire information on-line, you will be prompted to print the completed report form. Please sign the printed report form and return it to IRMM by fax (+32 14 571 865) or E-mail (JRC-IRMM-IMEP@ec.europa.eu). Check your results carefully before submission, since this is your final confirmation.

The deadline for submission of results is **22 October 2010**. It will not be possible to submit your results after the deadline.

Please also note that all communications during the comparative testing round should be directed to:

Diana Charels
E-mail: mbg-comparative-testing@jrc.ec.europa.eu
Phone: +39 0332 78 6518
Cc to: JRC-IRMM-IMEP@ec.europa.eu

We thank you very much for the collaboration in this comparative testing round.

Yours sincerely,

Guy Van den Eede
Head of Molecular Biology and Genomics Unit

Cc: G. Van den Eede, D. Charels, M. Mazzara.

15. Annex 3: Confirmation of shipment

Dear participant,

All test items for the second round of comparative testing have left the premises of IRMM (Geel, Belgium) this week. The parcel with test items that you will or have already received should contain:

- An acknowledgement of reception form, that should be returned to the EURL-GMFF by fax (+39 0332 789333). Should you encounter any problem with the shipment,

do not hesitate to contact Brigitte Fontenelle (brigitte.fontenelle@ec.europa.eu; phone +32 14 571 914),

- An accompanying letter entitled '**Participation in ILC-CRL-GMFF-CT-02/10, a comparative testing round to quantify the GM content of maize MON 810 test items'**.

The accompanying letter contains your **personal password** for on-line submission of your results to the reporting website https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do
Please find herewith a pdf file of the questionnaire. This pdf file is intended as an aid in the laboratory. In the questionnaire, items with the indication (number) behind the answer box indicate that a numerical value should be given. Items bearing a question mark icon on the right-hand side contain valuable and important information for the participant. In the reporting website clicking on the icon will give access to this information. Pdf files of questionnaires bearing hand-written answers **will not be accepted**. Only results and answers to the questionnaire reported on-line to the reporting website https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do will be accepted.

The deadline for submission of your results is **22 October 2010**.

Please send an E-mail to Maria-Maddalena.CHESSA@ec.europa.eu in case you have not received the above-mentioned documents. Thank you.

Kind Regards,
Maddalena Chessa on behalf of Diana Charels

European Commission - Joint Research Centre
Institute for Health and Consumer Protection
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16. Annex 4: Acknowledgement of receipt

FAX - Record for Quality System

JRC.I.4 -MV

Date: **R71GP6/EURL** 01/01/2009 **Acknowledgement of reception**
Page 1/1

Revision. c

From :

Lab Code:

To : Molecular Biology and Genomics Unit **fax: +39 0 332 78 9333**
Method Validation / EURL-GMFF
European Commission - Joint Research Centre - IHCP
21027 ISPRA (VA) Italy File nb EURL-CT-02/10

We have received the following samples	**In good condition** Yes	No
Two brown glass bottles containing maize powder		

Comments:

Date:..... Visa:.....

Please, send this document via FAX to:
+39 0332 78 9333 the day of reception

This document is not a recognition of the quantity and/or quality of samples and reagents provided. This document will be
used by EURL-GMFF only to confirm the reception of goods provided to participating laboratories in its Quality System.
EURL-GMFF thanks you very much for your participation.

European Commission

EUR 25028 **EN – Joint Research Centre – Institute for Health and Consumer Protection**
Title: Comparative Testing Report on the Detection and Quantification of Maize Event MON 810 - Comparative testing round: ILC-CRL-GMFF-CT-02/10
Author(s): D. Charels, T. Weber, M. Maras, M. Mazzara, C. Charles Delobel, C. Savini, G. Van den Eede
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Abstract
In the frame of Regulation (EC) No 882/2004, the European Union Reference Laboratory for Genetically Modified Food and Feed has the duty to organise comparative testing rounds and to ensure an appropriate follow-up of these activities. This report describes the outcome of the second comparative testing round ILC-CRL-GMFF-CT-02/10. Participants had to determine the GM content in two test items denoted maize powder levels 1 and 2, containing different GM percentages of maize event MON 810.
This comparative testing round was organised in collaboration with the Reference Materials Unit and the Food Safety and Quality Unit of the Institute for Reference Materials and Measurements (Geel, BE). The maize event MON 810 test items were produced by the Reference Materials Unit. The Food Safety and Quality Unit managed the on-line registration and submission of results.
A total of 136 laboratories were invited to participate in ILC-CRL-GMFF-CT-02/10. Six National Reference Laboratories declined participation, of which two were no longer a National Reference Laboratory. Ninety laboratories from 41 countries returned results, of which 65 were National Reference Laboratories, six were members of the European Network of GMO Laboratories only and 19 were laboratories from third countries. Two National Reference Laboratories, two Official control laboratories and nine laboratories from a third country did not submit any results.
Participants could report the results of the exercise either in mass/mass % or in copy/copy %.
The outcome of this second comparative testing round was in general positive, with 82-100 % of participants gaining a z-score in the range of -2 to +2 for both maize powder levels 1 and 2 regardless of the calibration method, the measurement unit and the approach used for calculating the z-score.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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