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Comparative Testing Report on the Quantification of Maize Line DAS-59122-7 and Oilseed rape Line GT73 (RT73)

Comparative testing round: ILC-EURL-GMFF-CT-01/12

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EURL
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for GM Food & Feed

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Joint Research Centre

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

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Confidentiality statement: The laboratory codes assigned to each participant in this comparative testing round are confidential. However, the EU-RL 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.

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Executive Summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF), established by Regulation (EC) No 1829/2003[(1)], organised a comparative testing round for National Reference Laboratories (NRLs) nominated under Regulation (EC) No 882/2004[(2)] and Regulation (EC) No 1981/2006[(3)], for members of the European Network of GMO Laboratories (ENGL), for Official control laboratories and for laboratories from third countries which had 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 EU-RL GMFF shall organise comparative testing and shall ensure an appropriate follow-up of such testing.

The design and execution of the comparative testing round were in accordance with the ISO 17043 Standard[(4)]. The EU-RL GMFF is accredited according to the ISO 17043 Standard 'General requirements for proficiency testing'[(4)].

The test items used in the comparative testing round ILC-EURL-GMFF-CT-01/12 were produced in-house. Monsanto Company provided dried leaves of GT73 also called RT73, (unique identifier MON-ØØØ73-7) oilseed rape. Pioneer Overseas Corporation provided dried leaves of DAS-59122-7 maize (unique identifier DAS-59122-7). DNA was extracted from leaves using a CTAB-based DNA extraction method. Participants had to determine the content of oilseed rape event GT73 and maize event 59122 in two test items denoted genomic DNA levels 1 and 2, containing different GM percentages of both GM events. In April 2012, a total of 160 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/12. Test items were shipped to participants in dry ice in the beginning of June 2012 in screw-cap tubes containing 400 µL of a genomic DNA solution at a concentration of 80 ng/uL. The Food Safety and Quality (FSQ) Unit of the Institute for Reference Materials and Measurements (IRMM) managed the on-line registration and submission of results. Eighty laboratories from 36 countries returned results, which fell into the following groups:

1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1),
2. 26 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
3. 30 were NRLs nominated under both Regulations (group 3),
4. 6 were only ENGL members (group 4),
5. 3 were only official control laboratories (group 5),
6. 12 were laboratories from third countries (group 6).

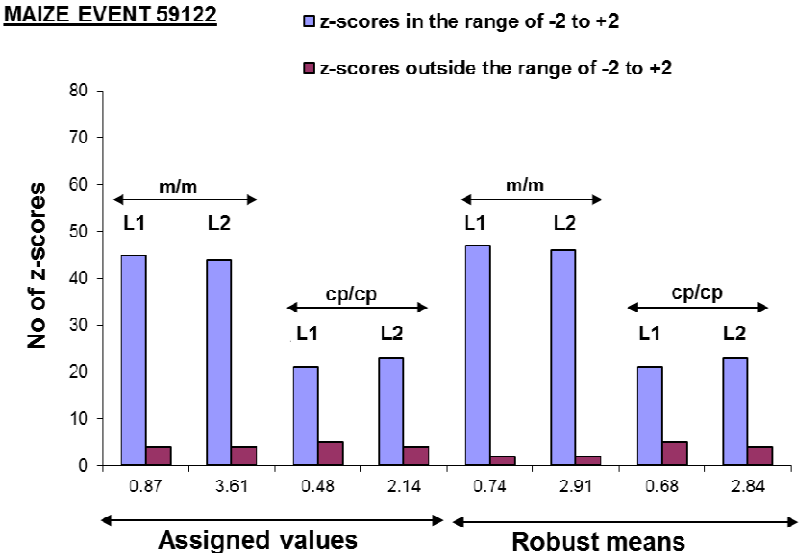
One NRL submitted one set of results but was appointed under Regulation (EC) No 882/2004 for one Member State and acting on behalf of another Member State as NRL appointed under Regulation (EC) No 1981/2006. Hence this laboratory is counted twice. One ENGL only member (group 4) registered twice but submitted both sets of results in the same measurement unit. One NRL (group 3) only submitted results when the participants with z-scores outside the range of -2 to +2 were repeating the experimental work. Five laboratories including one NRL (group 3) and four laboratories from third countries (group 6) did not

submit results. The FSQ Unit of IRMM managed the on-line registration and submission of results.

Participants could report the results in either mass/mass % (m/m %) or copy/copy % (cp/cp %). The EU-RL GMFF calculated the robust means (μ_R) of the genomic DNA levels 1 and 2 test items in m/m % and in cp/cp %. All data were log-transformed and then robust statistics were applied to obtain a robust mean [5, 6, 7]. In addition, values (μ) were assigned by the EU-RL GMFF on the basis of the data from the homogeneity study[8] (m/m % data) and digital Polymerase Chain Reaction[9] (cp/cp % data). The homogeneity, stability and digital Polymerase Chain Reaction studies were conducted at the EU-RL GMFF. These data were included in the uncertainty budget.

The target standard deviation for comparative testing $\hat{\sigma}$ was fixed at 0.20 ($\log_{10}$ value) for maize event 59122 and oilseed rape line GT73 by the Advisory Board for Comparative testing. This target standard deviation was used to derive z-scores for the participants' results. An overview of the robust means and number of z-scores in the range of -2 to +2 is given in Figure 1.

a)



b)

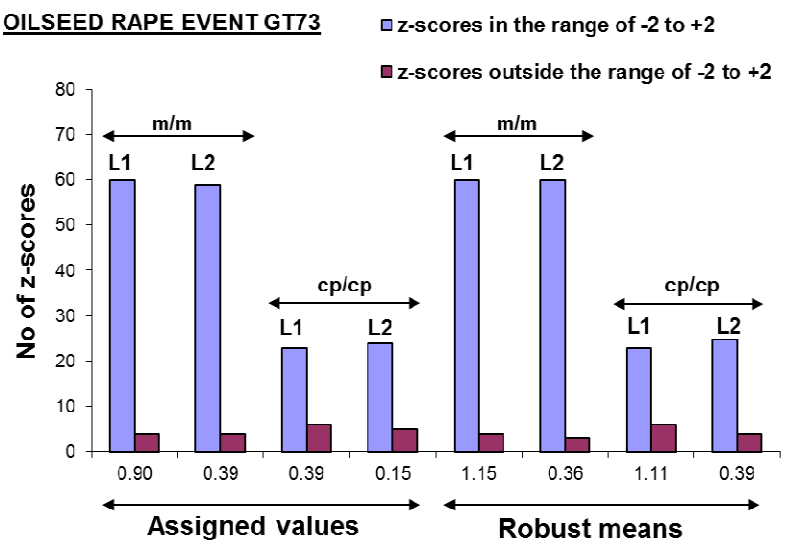


Figure 1. Overview of z-scores calculated on the basis of assigned values and robust means for maize event 59122 (a) and oilseed rape event GT73 (b). m/m = results submitted in mass/mass %, cp/cp = results submitted in copy/copy %, L1 = level 1, L2 = level 2.

In this fifth comparative testing round 92 % to 98 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in m/m % depending on the GM content and the GM event. However, a lower percentage (38 – 93 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in cp/cp %.

Participants' assessment of results in relation to measurement uncertainty needs to be improved because about 53 % of participants provided information on measurement uncertainty in a complete and consistent manner.

Contents

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1. Introduction

The Joint Research Centre (JRC) as EU-RL GMFF was established by Regulation (EC) No 1829/2003[1]. The EU-RL GMFF has two mandates determined by Regulation (EC) No 1981/2006[3] and by Regulation (EC) No 882/2004[2].

In accordance with Article 32 of Regulation (EC) No 882/2004 the EU-RL GMFF shall organise comparative testing for 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 '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 which is used by the Member States of the European Union in the official control of food and feed. Hence, an accurate determination of the GM content is of paramount importance.

In 2012 the EU-RL GMFF organised the fifth comparative testing round in collaboration with the FSQ Unit of IRMM. The comparative testing round was announced in an official communication sent to all NRLs and ENGL members on the 21st of December 2011. In April 2012, a total of 160 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/12, and subsequently 85 laboratories registered for this comparative testing round. Test items were shipped to participants in dry ice in the beginning of June 2012 in screw-cap tubes containing 400 µL of a genomic DNA solution at a concentration of 80 ng/uL. Eighty laboratories from 36 countries returned results, which fell into the following groups:

1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1),
2. 26 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
3. 30 were NRLs nominated under both Regulations (group 3),
4. 6 were only ENGL members (group 4),
5. 3 were only official control laboratories (group 5),
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One NRL submitted one set of results but was appointed under Regulation (EC) No 882/2004 for one Member State and acting on behalf of another Member State as NRL appointed under Regulation (EC) No 1981/2006. Hence this laboratory is counted twice. One ENGL member only (group 4) registered twice but submitted both sets of results in the same measurement unit. One NRL (group 3) only submitted results when the participants with z-scores outside the range of -2 to +2 were repeating the experimental work. Five laboratories including one NRL (group 3) and four laboratories from third countries (group 6) did not submit results. The FSQ Unit of IRMM managed the on-line registration and submission of results.

2. Description of comparative test items

2.1 Preparation

Test items were prepared in house in accordance with ISO Guide 34[10] regarding the 'General requirements for the competence of reference material producers'.

Maize and oilseed rape DNA levels 1 and 2 were prepared to nominal values of 0.6 m/m % and 2.6 m/m % GM of 59122, 1.1 m/m % and 0.4 m/m % GM of GT73, respectively.

The preparation of test items was carried out between March and May 2012. Test items consisted of DNA extracted from ground non-GM grains of maize and oilseed rape, and ground leaf material of 59122 maize and GT73 oilseed rape using a CTAB-based method. Powder of non-GM maize used in DNA extraction was prepared by a one-step grinding process using an Ultra Centrifugal Mill ZM200 (Retsch GmbH, DE), while the powder of non-GM oilseed rape and both GM events was prepared by knife mill Grindomix GM200 (Retsch GmbH, DE). Every DNA extract included in final test items was assessed for the presence of other GM events authorised within the European Union. The zygosity of events 59122 and GT73 was individually assessed in the single GM events.

Test items were prepared in a one-step dilution by mixing DNA of non-modified maize and oilseed rape with DNA of 59122 maize and GT73 oilseed rape in specified proportions.

Approximately 400 µL of the test items were aliquoted in 2-mL screw cap microcentrifuge tubes. Tubes were labelled according to the GM level of the test items and stored at -20 ºC.

2.2 Purity testing

Purity tests conducted at the EU-RL GMFF detected only DNA of the GM events included in this comparative testing round.

2.3 Homogeneity and stability assessment

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

Samples are considered to be adequately homogeneous if:

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

Where s_s is the between-test item standard deviation 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-test item standard deviation contributes no more than about 10 % to the standard deviation for comparative testing.
The repeatability of the test method is the square root of mean sum of squares within-test item MS_{within}. The relative between-test item standard deviation $s_{s,rel}$ is given by

$$s_{s,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \times 100\% \quad (2)$$

where: $MS_{between}$ is the mean sum of squares between test items
MS_{within} is the mean sum of squares within test items
n is the number of replicates
$\bar{y}$ is the mean of the homogeneity data

If $MS_{within} > MS_{between}$, then:

$$s_{s,rel} = u^*_{bb} = \frac{\frac{repeatability}{\sqrt{n}} \sqrt[4]{\frac{2}{N(n-1)}}}{\bar{y}} \times 100\% \quad (3)$$

where: u^*_{bb} is the maximum uncertainty contribution that can be obtained by the hidden heterogeneity of the material.

For each level and GM event ten test items ($N = 10$) were randomly selected and analysed in three-fold replicates ($n = 3$). The criterion described in formula (1) was fulfilled thus indicating that both genomic DNA levels 1 and 2 test items were homogeneous.
The data from the homogeneity study conducted at the EU-RL GMFF were used for the estimation of the uncertainty contributions related to the homogeneity of the genomic DNA levels 1 and 2 test items, respectively.

An isochronous short term stability study involving two test items from level 1 only ($N = 2$, $n = 3$), was conducted over time periods of one, two and four weeks at temperatures of +4 ºC and +18 ºC[13]. The results of the study did not reveal any influence of time and temperature on the stability of test items. Because of the shipment of a genomic DNA solution, it was decided to ship test items in dry ice to participants.

An isochronous long term stability study involving two genomic DNA level 1 test items ($N = 2$, $n = 3$) was conducted for time periods of three, six and eight months at a temperature of -20 ºC[13]. No significant trend (95 % confidence level) was detected for any of the GM events tested thus indicating that test items can be stored at -20 ºC.

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

Participants had to determine the content of maize event 59122 and oilseed rape event GT73 in two test items denoted genomic DNA levels 1 and 2, containing different GM percentages of both GM events. Participants could report the quantitative results in either m/m % or 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.

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

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

A total of 80 laboratories from 36 countries reported results (Figures 2 and 3).

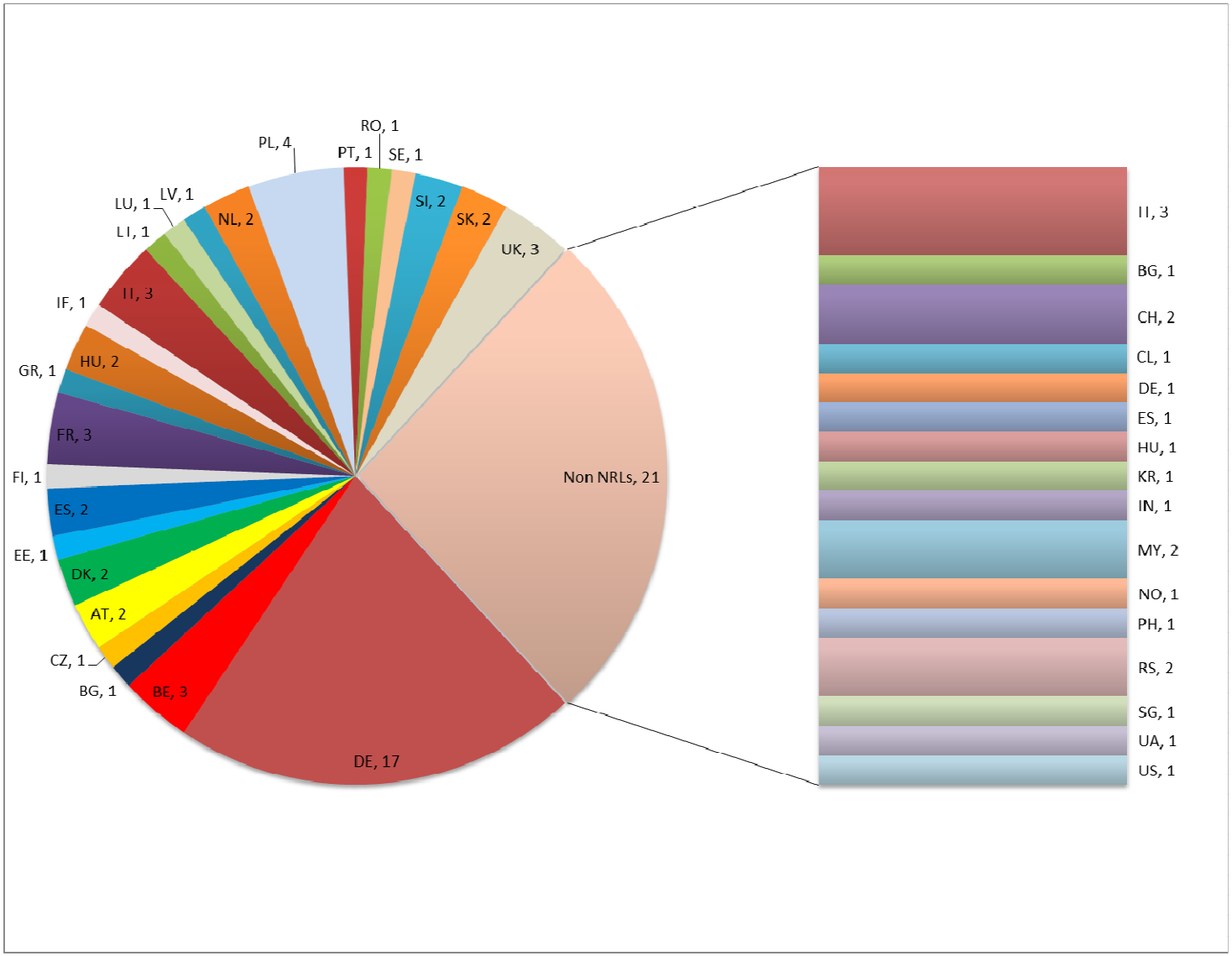

Figure 2: Distribution of participants from different countries.

A majority of laboratories reported the GM content in m/m % (Figure 4). A minority of laboratories expressed their results in cp/cp % (Figure 4) of which one laboratory (L01) used a dual target plasmid and one laboratory (L79) used a single target plasmid. All other laboratories used a genomic DNA calibrant: Certified Reference Material (CRM) from IRMM (maize event 59122) and AOCS (oilseed rape GT73) or a commercial kit. One laboratory registered twice (L08 and L74) and submitted both sets of results in cp/cp % (Figure 4).

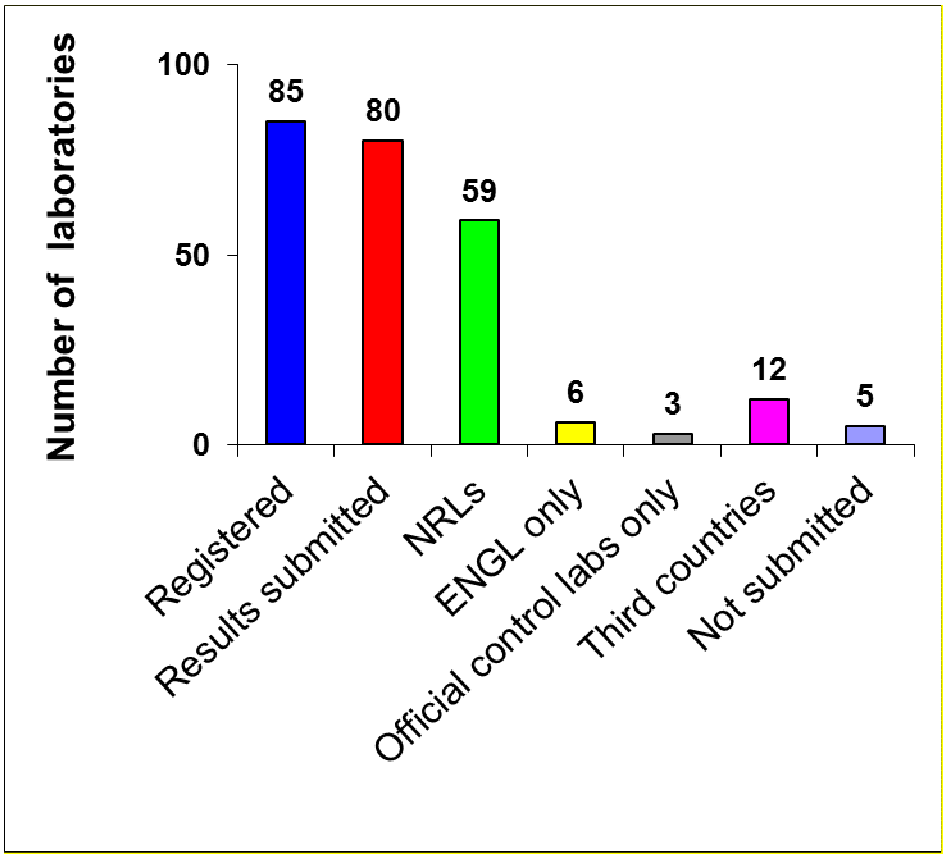

Figure 3. Overview of participants' results grouped by type of laboratory.

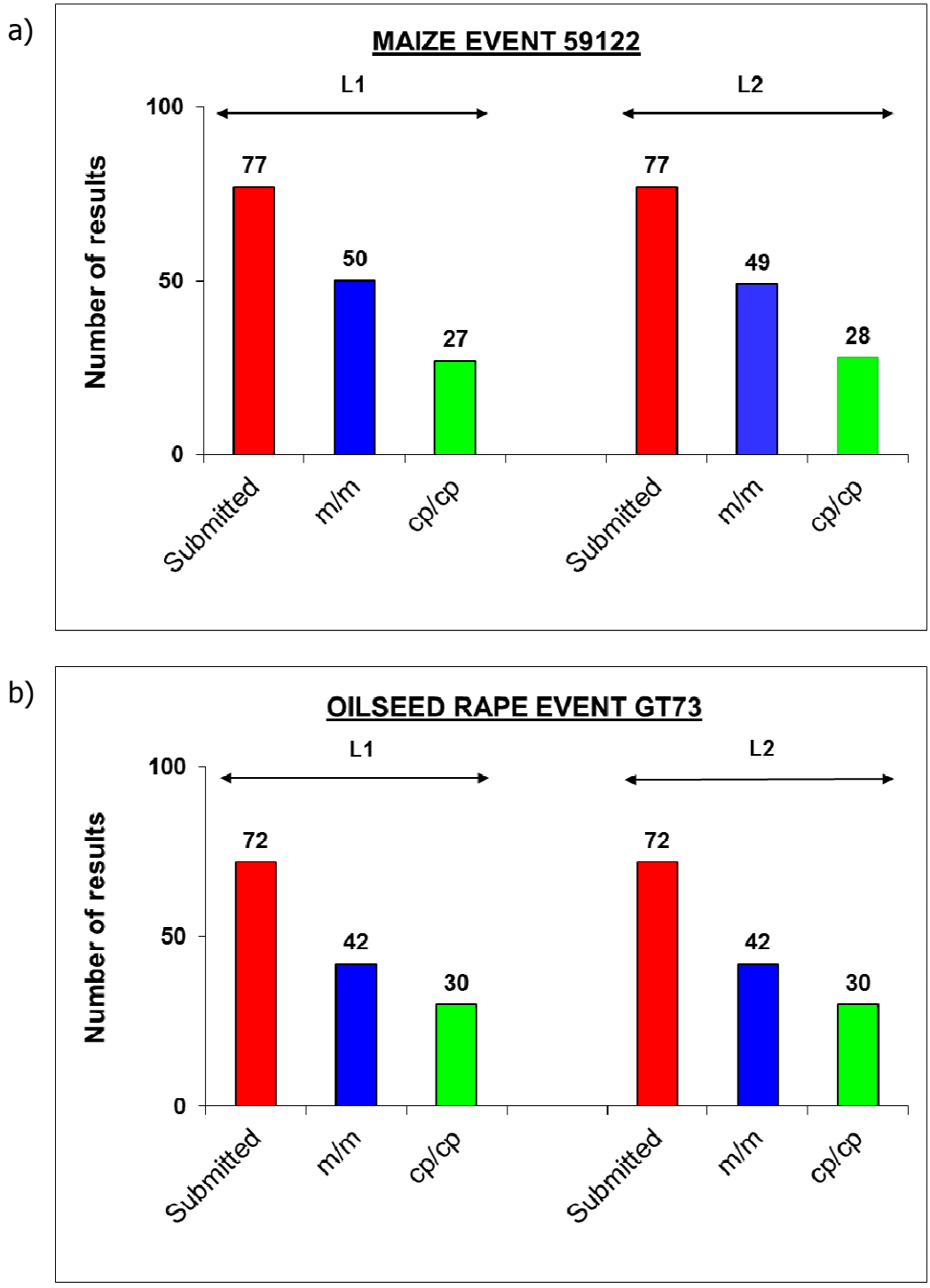

Figure 4. Overview of participants' results grouped by GM event maize 59122 (a), oilseed rape GT73 (b) and measurement unit. m/m = results submitted in mass/mass %, cp/cp = results submitted in copy/copy %, L1 = level 1, L2 = level 2. Note: For maize event 59122 L84 reported in m/m for L1 and cp/cp for L2.

The EU-RL GMFF calculated the robust means (μ_R) of the genomic DNA 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[5, 6, 7]. In addition, values (μ) were assigned by the EU-RL GMFF on the basis of the data from the homogeneity study (m/m % data) and digital Polymerase Chain Reaction (cp/cp % data).

Data from the homogeneity and stability studies conducted by the EU-RL GMFF were included in the uncertainty budget.

An overview of the results reported in m/m % and cp/cp % is given in Tables 3 to 16. An overview of the analytical methods used by each participant is summarised in section 11 'Questionnaire data'.

4. Assigned value and measurement uncertainty

4.1 Reference values determined by the test item producer

Following evaluation of the data it was decided to include reference values determined by the EU-RL GMFF in this, the final report. This was because of the large discrepancy between the robust means based on the participants' results expressed in cp/cp % and the data from the in-house digital PCR experiments already described in the preliminary report. The assigned value in m/m % (μ) is derived from the homogeneity data ($N = 10$, $n = 3$)[8]. The assigned value in cp/cp % (μ) was determined by digital PCR ($N = 5$, $n = 5$)[9].

The information relating to the EURL-GMFF-CT-01/12 genomic DNA level 1 and 2 test items is outlined in the Table below.

Table 1. Assigned value (μ) and expanded uncertainty of genomic DNA levels 1 and 2. [1] Relative standard uncertainty relating to the characterisation, [2] Relative standard uncertainty resulting from the homogeneity assessment, [3] Relative standard uncertainty resulting from the long-term stability assessment

μ [m/m %]		Expanded uncertainty ($U = 2 * u_c$)		Relative standard uncertainty contributions [%]		
		U_{abs} [m/m %]	U_{rel} [%]	$(u_{char, rel})^1$	$(u_{bb, rel})^2$	$(u_{lts, rel})^3$
Maize 59122						
Level 1	0.87	0.31	36	6.13	4.17	16.16
Level 2	3.61	1.32	37	5.93	6.37	16.16
Oilseed rape GT73						
Level 1	0.90	0.15	17	3.52	6.86	3.43
Level 2	0.39	0.10	25	10.42	6.04	3.43
μ [cp/cp %]		**U_{abs} [cp/cp %]**	**U_{rel} [%]**			
Maize 59122						
Level 1	0.48	0.16	34	3.89	4.17	16.16
Level 2	2.14	0.82	38	7.97	6.37	16.16
Oilseed rape GT73						
Level 1	0.39	0.08	20	6.76	6.86	3.43
Level 2	0.15	0.03	23	9.37	6.04	3.43

The expanded uncertainty (U) comprises standard uncertainty contributions from the characterisation of the material (u_{char}), the between-test item homogeneity (u_{bb}) and the long-term stability of the material (u_{lts})[15]. The uncertainty contribution from the characterisation of the material is calculated using formula (7). A coverage factor of 2 was used to calculate the expanded uncertainty corresponding to a 95 % level of confidence[16].

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

The standard uncertainty (u_{char}) of the characterisation is calculated using the formula:

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

where: σ = relative standard deviation of the mean
N = number of data points.

The assigned values of genomic DNA 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.

The assigned values of genomic DNA levels 1 and 2 expressed in cp/cp % are traceable to the digital PCR method that was used to determine the GM content.

4.2 Consensus values from participants

The consensus value (μ_R) from participants in the comparative testing round was calculated using robust statistics[17]. 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[6]. Robust means (μ_R) were calculated on the basis of the results reported in m/m % and cp/cp %, respectively.

The expanded uncertainty (U) comprises standard uncertainty contributions from the characterisation, the between-test item homogeneity, and the stability[15] (Formula 6).

The robust means (μ_R) determined by the EU-RL GMFF are depicted in Table 2.

Table 2. Overview of robust means (μ_R) and expanded uncertainties for genomic DNA levels 1 and 2

μ_R [m/m %]	Expanded uncertainty ($U = 2 * u_c$)	
	U_{abs} [m/m %]	U_{rel} [%]
Maize 59122		
Level 1 0.74 (N = 49)	0.26	35
Level 2 2.91 (N = 48)	1.04	36
Oilseed rape GT73		
Level 1 1.15 (N = 41)	0.18	17
Level 2 0.36 (N = 40)	0.06	17
μ_R [cp/cp %]	U_{abs} [cp/cp %]	U_{rel} [%]
Maize 59122		
Level 1 0.68 (N = 26)	0.27	40
Level 2 2.84 (N = 27)	1.18	40
Oilseed rape GT73		
Level 1 1.11 (N = 29)	0.22	20
Level 2 0.39 (N = 29)	0.08	22

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 assigned values[8, 9] (μ) and the robust means[17] (μ_R) of the participants' results.
Laboratories are compared on the basis of z-scores calculated from log-transformed data[6]. The z-scores are based on the assigned values (μ) and the robust means (μ_R) of the submitted results (Tables 3 to 16). Participants reported results in m/m % and/or cp/cp %. All results reported in cp/cp % were pooled irrespective of the DNA calibrant used (i.e. plasmid or genomic DNA) due to the limited number of results obtained with a plasmid DNA calibrant (N = 2).

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 values of 0.20 for the maize event 59122 and oilseep rape event GT73[18]. The z-score (z_i) for participant i reporting measurement result x_i is thus calculated as

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

where: μ = assigned value

$$z_i = \left(\log_{10} x_i - \log_{10} \mu_R \right) \Big/ \hat{\sigma} \qquad (9)$$

where: μ_R = robust mean

Table 3. z-scores for maize event 59122 genomic DNA level 1 (L1) for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (e) *U* seems to be overestimated, (f) *U* seems to be underestimated. L84 reported in m/m % for L1 and cp/cp % for genomic DNA level 2 (L2). Results are as submitted by participants.

Maize event 59122 Robust mean = 0.74 m/m % Assigned value = 0.87 m/m %							
Laboratory number	Value	Uncertainty		LOD m/m	LOQ m/m	z-score[1]	z-score[2]
		relative	absolute				
L02	**0.97**	(b) 30.00		0.10	0.10	**0.59**	**0.24**
L03	**0.87**		0.25	-	0.10	**0.35**	**0.00**
L05	**0.92**	(c) 0.36		0.05	0.10	**0.47**	**0.12**
L06	**1.13**		0.54	0.10	0.30	**0.92**	**0.57**
L07	**0.63**	43.2		0.10	-	**-0.35**	**-0.70**
L10	**0.78**	(c) 0.16		-	-	**0.11**	**-0.24**
L11	**1.31**		0.25	0.03	0.10	**1.24**	**0.89**
L12	**0.69**		0.28	0.02	0.20	**-0.14**	**-0.49**
L15	**0.57**	(c) 0.24		0.03	0.06	**-0.57**	**-0.92**
L16	**0.93**		(a) 0.24	0.10	0.50	**0.49**	**0.14**
L17	**0.46**		0.14	0.02	0.10	**-1.03**	**-1.38**
L18	**0.84**		0.23	0.07	0.30	**0.27**	**-0.08**
L19	**1.15**		(a) 0.27	-	0.05	**0.96**	**0.61**
L21	**0.84**	40.00		0.10	0.10	**0.27**	**-0.08**
L23	**0.32**	(c) 0.12		0.02	0.10	**-1.82**	**-2.17**
L24	**0.97**	30.00		0.10	0.10	**0.59**	**0.24**
L25	**0.71**		0.10	<0.10	0.10	**-0.09**	**-0.44**
L26	**0.81**	40.88		-	-	**0.19**	**-0.16**
L27	**0.72**		0.21	-	-	**-0.06**	**-0.41**
L28	**0.56**	(c) 0.10		0.06	0.11	**-0.61**	**-0.96**
L29	**3.76**	(c) 0.30		0.04	0.10	**3.53**	**3.18**
L31	**0.60**	(a) (c) 0.20		0.01	0.10	**-0.46**	**-0.81**
L32	**0.62**	(a)(b)(c) 0.11		0.20	0.10	**-0.39**	**-0.74**
L34	**0.61**		0.07	0.03	0.10	**-0.42**	**-0.77**
L37	**0.63**	18.72		-	0.10	**-0.35**	**-0.70**
L38	**0.90**	6.30		-	-	**0.42**	**0.07**
L39	**0.60**	34.00		0.04	0.10	**-0.46**	**-0.81**
L41	**0.75**	(b) (c) 0.08		0.04	0.09	**0.03**	**-0.32**
L43	**0.62**	-	-	0.05	0.10	**-0.39**	**-0.74**
L44	**0.67**	50.00		0.04	0.09	**-0.22**	**-0.57**
L47	**0.90**		0.20	-	0.05	**0.42**	**0.07**
L50	**0.49**	(b) (c) 0.15		0.02	0.10	**-0.90**	**-1.25**
L51	**0.55**		(a) 0.20	-	0.05	**-0.65**	**-1.00**
L52	**>0.10**	-	-	0.10	-	*****	*****
L54	**0.69**		0.07	0.005	0.01	**-0.15**	**-0.50**
L56	**0.56**		0.21	<0.1	0.10	**-0.63**	**-0.98**
L57	**0.36**	(c) 0.06		0.10	0.10	**-1.57**	**-1.92**
L60	**1.24**		0.29	0.01	0.10	**1.12**	**0.77**
L63	**1.32**	60.00		-	-	**1.25**	**0.91**
L64	**0.53**		(f) 0.035	0.01	0.10	**-0.73**	**-1.08**
L65	**1.22**		0.46	0.02	0.10	**1.08**	**0.73**
L66	**0.73**	-	-	-	-	**-0.03**	**-0.38**
L68	**0.75**		0.23	0.03	0.20	**0.03**	**-0.32**
L69	**0.75**		(a) (b) 0.06	0.05	0.10	**0.03**	**-0.32**
L71	**0.32**	(a) 0.1		0.01	0.10	**-1.82**	**-2.17**
L72	**0.52**		0.13	0.01	0.05	**-0.77**	**-1.12**
L75	**0.64**		(a) 0.25	0.05	0.17	**-0.32**	**-0.67**
L81	**0.76**	(c) 0.20		0.04	0.09	**0.06**	**-0.29**
L84	**0.83**	-	-	0.05	0.10	**0.25**	**-0.10**
L86	**3.32**	(e) 116.89		0.03	0.10	**3.26**	**2.91**

Table 4. z-scores for maize event 59122 genomic DNA level 2 (L2) for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (e) *U* seems to be overestimated, (f) *U* seems to be underestimated. Results are as submitted by participants.

Maize event 59122							
	Robust mean = 2.91 m/m %						
Laboratory	Assigned value = 3.61 m/m %						
number	Value	Uncertainty		LOD m/m	LOQ m/m	z-score[1]	z-score[2]
		relative	absolute				
L02	3.34	(b) 30.00		0.10	0.10	0.30	-0.17
L03	2.37		0.66	-	0.10	-0.44	-0.91
L05	0.34	(c) 0.13		0.05	0.10	-4.66	-5.13
L06	4.68		1.22	0.10	0.30	1.04	0.56
L07	2.59	38.10		0.10	-	-0.25	-0.72
L10	3.04	(c) 0.59		-	-	0.10	-0.37
L11	4.82		1.40	0.03	0.10	1.10	0.63
L12	2.75		0.86	0.02	0.20	-0.12	-0.59
L15	2.27	(c) 0.94		0.03	0.06	-0.54	-1.01
L16	3.62		(a) 0.48	0.10	0.50	0.48	0.01
L17	2.63		0.79	0.02	0.10	-0.22	-0.69
L18	3.16		0.78	0.07	0.30	0.18	-0.29
L19	3.82		(a) 0.91	-	0.05	0.59	0.12
L21	3.64	40.00		0.10	0.10	0.49	0.02
L23	1.83	(c) 0.46		0.02	0.10	-1.00	-1.48
L24	3.22	30.00		0.10	0.10	0.22	-0.25
L25	2.86		0.35	<0.10	0.10	-0.03	-0.51
L26	2.83	36.25		-	-	-0.06	-0.53
L27	2.87		0.70	-	-	-0.03	-0.50
L28	2.81	(c) 0.53		0.07	0.12	-0.07	-0.54
L29	11.04	(c) 0.45		0.04	0.10	2.90	2.43
L31	3.10		(a) 0.90	0.01	0.10	0.14	-0.33
L32	2.52	(a)(b)(c) 0.58		0.20	0.10	-0.31	-0.78
L34	2.83		0.17	0.03	0.10	-0.06	-0.53
L37	2.48	14.66		-	0.10	-0.34	-0.82
L38	4.00	6.30		-	-	0.69	0.22
L39	2.60	27.00		0.04	0.10	-0.24	-0.71
L41	3.30	(b) (c) 0.34		0.04	0.09	0.28	-0.19
L43	2.94	-	-	0.05	0.10	0.03	-0.45
L44	3.19	50.00		0.04	0.09	0.20	-0.27
L47	3.50		0.70	-	0.05	0.40	-0.07
L50	2.46	(b) (c) 0.74		0.02	0.10	-0.36	-0.83
L51	1.78		(a) 0.20	-	0.05	-1.06	-1.54
L52	>0.10	-	-	0.10	-	*	*
L54	3.41		0.25	0.01	0.01	0.35	-0.12
L56	2.48		0.62	<0.10	0.10	-0.35	-0.82
L57	1.47	(c) 0.10		0.10	0.10	-1.48	-1.95
L60	6.72		1.55	0.01	0.10	1.82	1.35
L63	4.87	28.00		-	-	1.12	0.65
L64	2.00		(f) 0.01	0.01	0.10	-0.81	-1.28
L65	6.04		1.32	0.02	0.10	1.59	1.12
L66	2.81	-	-	-	-	-0.07	-0.54
L68	3.11		0.76	0.03	0.20	0.15	-0.32
L69	2.99		(a) (b) 0.40	0.05	0.10	0.06	-0.41
L71	1.25		(a) 0.40	0.01	0.10	-1.83	-2.30
L72	2.50					-0.33	-0.80
L75	2.85		(a) 0.66	0.05	0.17	-0.04	-0.51
L81	2.89	(c) 0.30		0.04	0.09	-0.01	-0.48
L86	1.27	(e) 247.60		0.03	0.10	-1.80	-2.27

Table 5. z-scores for maize event 59122 genomic DNA level 1 (L1) for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean is reported for information purpose only, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value(e) *U* seems to be overestimated, (g) reported in m/m %, (h) seems to be reported in absolute copy numbers. Results are as submitted by participants.

Maize event 59122							
	Robust mean = *0.68 cp/cp %*						
Laboratory number	Assigned value = 0.48 cp/cp %						
	Value	Uncertainty		LOD cp/cp	LOQ cp/cp	z-score[1]	z-score[2]
		relative	absolute				
L01	**0.21**	(b) 13.00		0.03	0.06	***-2.56***	**-1.82**
L08	**0.74**	-	-	(g) 0.10	(g) 0.10	***0.18***	**0.92**
L13	**0.93**		0.18	(h) 14.00	(g) 0.10	***0.67***	**1.42**
L14	**0.87**	29.32		0.02	0.05	***0.53***	**1.27**
L20	**1.10**	(b) 10.00		0.05	0.10	***1.04***	**1.78**
L30	**0.39**	(b) 27.00		-	-	***-1.21***	**-0.47**
L35	**2.50**		(a) 0.70	0.10	0.50	***2.82***	**3.56**
L36	**0.98**		0.30	0.10	0.20	***0.79***	**1.53**
L40	**0.30**		(d) 18.34	-	-	***-1.78***	**-1.04**
L42	**0.75**	-	-	0.01	0.10	***0.21***	**0.95**
L45	**0.46**	26.72		0.05	0.10	***-0.85***	**-0.11**
L46	**0.32**		0.13	-	-	***-1.64***	**-0.90**
L53	**0.76**		(b) 0.07	(h) 6.00	(h) 11.00	***0.24***	**0.98**
L55	**0.34**	(c) 0.08		0.015	0.12	***-1.51***	**-0.77**
L58	**1.42**	(a) 21.30		0.10	0.09	***1.59***	**2.33**
L59	**1.95**		0.31	0.05	0.10	***2.28***	**3.02**
L61	**0.56**	77.00		0.06	0.30	***-0.42***	**0.33**
L62	**0.42**	-	-	-	-	***-1.05***	**-0.31**
L73	**0.85**		0.11	-	0.07	***0.48***	**1.22**
L74	**0.60**	-	-	(g) 0.10	(g) 0.10	***-0.28***	**0.46**
L76	**0.74**	(c) 0.10		0.10	0.10	***0.18***	**0.92**
L77	**1.00**		0.39	-	-	***0.83***	**1.57**
L78	**0.69**	23.10		0.01	0.10	***0.03***	**0.77**
L79	**0.15**	12.23		0.01	0.01	***-3.29***	**-2.55**
L82	**0.65**		0.20	0.05	0.10	***-0.10***	**0.64**
L85	**2.03**		1.05	0.09	0.25	***2.37***	**3.11**
L87	**>0.10**	-	-	0.10	0.10	*	*

Table 6. z-scores for maize event 59122 genomic DNA level 2 (L2) for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean is reported for information purpose only, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (f) *U* seems to be underestimated, (g) reported in m/m %, (h) seems to be reported in absolute copy numbers. L84 reported in m/m % for genomic DNA level 1 (L1) and cp/cp % for L2. Results are as submitted by participants.

Maize event 59122							
	Robust mean = *2.84 cp/cp %*						
Laboratory	Assigned value = 2.14 cp/cp %						
number	**Value**	**Uncertainty**		**LOD cp/cp**	**LOQ cp/cp**	**z-score[1]**	**z-score[2]**
		relative	**absolute**				
L01	**0.91**	(b) 3.35		0.03	0.06	***-2.47***	**-1.86**
L08	**2.65**	-	-	(g) 0.10	(g) 0.10	***-0.15***	**0.47**
L13	**4.66**		0.18	(h) 14.00	(g) 0.10	***1.07***	**1.69**
L14	**3.16**	17.12		0.02	0.05	***0.23***	**0.85**
L20	**4.30**	(b) 10.00		0.05	0.10	***0.90***	**1.52**
L30	**1.52**	(b) 17.00		-	-	***-1.36***	**-0.74**
L35	**7.30**		(a) 0.60	0.10	0.50	***2.05***	**2.67**
L36	**4.50**		1.10	0.10	0.20	***1.00***	**1.62**
L40	**1.24**		(d) 21.89	-	-	***-1.80***	**-1.18**
L42	**2.56**	-	-	0.01	0.10	***-0.23***	**0.39**
L45	**2.13**	(f) 4.16		0.05	0.10	***-0.63***	**-0.01**
L46	**1.42**		0.47	-	-	***-1.51***	**-0.89**
L53	**2.31**		(b) (f) 0.12	(h) 6.00	(h) 11.00	***-0.45***	**0.17**
L55	**1.70**	(c) 0.52		0.02	0.12	***-1.11***	**-0.50**
L58	**6.62**	(a) 21.30		0.10	0.09	***1.84***	**2.45**
L59	**8.40**		1.23	0.05	0.10	***2.35***	**2.97**
L61	**3.01**	19.40		0.06	0.30	***0.12***	**0.74**
L62	**2.03**	-	-	-	-	***-0.73***	**-0.11**
L73	**3.79**		0.54	-	0.07	***0.63***	**1.24**
L74	**2.80**	-	-	(g) 0.10	(g) 0.10	***-0.03***	**0.59**
L76	**4.03**	(c) 0.29		0.10	0.10	***0.76***	**1.38**
L77	**2.80**		0.96	-	-	***-0.03***	**0.59**
L78	**2.66**	23.10		0.01	0.10	***-0.14***	**0.47**
L79	**0.87**	19.40		0.01	0.01	***-2.57***	**-1.95**
L82	**2.50**		0.60	0.05	0.10	***-0.28***	**0.34**
L84	**2.99**	-	-	(g) 0.05	(g) 0.10	***0.11***	**0.73**
L85	**6.45**		2.26	0.09	0.25	***1.78***	**2.40**
L87	**>0.10**	-	-	-	-	*****	*****

Table 7. z-scores for oilseed rape event GT73 genomic DNA level 1 (L1) for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, * = no z-score attributed, [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (f) *U* seems to be underestimated, (i) practical LOQ is in contradiction with the reported GM content. Results are as submitted by participants.

Oilseed event GT73							
	Robust mean = 1.15 m/m %						
Laboratory	Assigned value = 0.90 m/m %						
number	Value	Uncertainty		LOD m/m	LOQ m/m	z-score[1]	z-score[2]
		relative	absolute				
L02	**1.49**	30.00		0.08	0.10	**0.83**	**1.09**
L03	**1.02**		0.34	-	0.10	**0.00**	**0.27**
L05	**0.68**	(c) 0.22		0.05	0.10	**-0.88**	**-0.61**
L06	**1.06**		(f) 0.07	0.10	0.30	**0.09**	**0.36**
L10	**1.17**	(c) 0.20		-	-	**0.30**	**0.57**
L11	**0.73**		0.21	0.03	0.10	**-0.72**	**-0.45**
L12	**1.04**		0.54	0.007	0.07	**0.05**	**0.32**
L15	**0.78**	(c) 0.35		0.01	0.05	**-0.58**	**-0.31**
L17	**0.87**		0.31	0.02	0.10	**-0.34**	**-0.07**
L18	**0.91**		0.30	0.01	0.04	**-0.24**	**0.02**
L21	**0.85**	26.00		0.10	0.10	**-0.39**	**-0.12**
L23	**1.83**	(c) 0.59		0.05	0.10	**1.27**	**1.54**
L24	**1.47**	30.00		0.08	0.10	**0.80**	**1.07**
L25	**0.96**		0.43	<0.01	0.10	**-0.13**	**0.14**
L26	**1.12**	48.20		-	-	**0.21**	**0.47**
L27	**0.60**		0.21	-	-	**-1.15**	**-0.88**
L28	**0.99**	(c) 0.15		0.01	0.10	**-0.06**	**0.21**
L29	**0.86**	(c) (f) 0.05		0.03	0.10	**-0.37**	**-0.10**
L31	**1.00**		(a) 0.30	0.001	0.02	**-0.04**	**0.23**
L32	**1.50**	(a) (b) (c) 0.52		0.025	0.10	**0.84**	**1.11**
L34	**0.94**		0.11	0.01	0.03	**-0.17**	**0.09**
L37	**0.75**	24.72		-	0.10	**-0.66**	**-0.40**
L39	**0.83**	34.00		0.03	0.10	**-0.44**	**-0.18**
L41	**0.89**	(b) (c) 0.097		0.02	0.04	**-0.29**	**-0.02**
L43	**1.16**	-	-	0.05	0.10	**0.28**	**0.55**
L44	**1.13**	50.00		0.04	0.09	**0.23**	**0.49**
L50	**0.98**	(b) (c) 0.29		0.02	0.10	**-0.08**	**0.18**
L54	**1.06**		0.10	0.005	0.01	**0.09**	**0.36**
L56	**1.51**		0.34	<0.10	0.10	**0.85**	**1.12**
L57	**1.10**	(c) (f) 0.03		0.04	0.10	**0.17**	**0.44**
L60	**0.95**	(c) 0.28		0.01	0.10	**-0.15**	**0.12**
L63	**1.48**	24.00		-	-	**0.81**	**1.08**
L65	**1.01**		0.18	0.01	0.10	**-0.02**	**0.25**
L66	**3.24**	-	-	-	-	**2.51**	**2.78**
L67	**<3.51**	-	-	-	-	*****	*****
L68	**1.06**		0.21	0.01	0.35	**0.09**	**0.36**
L69	**0.77**		(a) 0.08	0.05	0.10	**-0.61**	**-0.34**
L71	**1.00**		(a) 0.32	0.01	0.10	**-0.04**	**0.23**
L72	**0.81**		0.16	0.01	0.06	**-0.50**	**-0.23**
L75	**0.96**		(a) 0.44	-	-	**-0.13**	**0.14**
L78	**0.93**	27.15		-	-	**-0.20**	**0.07**
L86	**1.34**	38.42		(i) 4.90	14.85	**0.60**	**0.86**

Table 8. z-scores for oilseed rape event GT73 genomic DNA level 2 (L2) for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, * = no z-score attributed, [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (f) *U* seems to be underestimated. Results are as submitted by participants.

Oilseed event GT73							
		Robust mean = 0.36 m/m %					
Laboratory		Assigned value = 0.39 m/m %					
number	Value	Uncertainty		LOD m/m	LOQ m/m	z-score[1]	z-score[2]
		relative	absolute				
L02	0.55	30.00		0.08	0.10	0.90	0.75
L03	0.42		0.14	-	0.10	0.31	0.16
L05	2.40	(c) 0.77		0.05	0.10	4.10	3.95
L06	0.38		0.08	0.10	0.30	0.10	-0.06
L10	0.43	(c) 0.10		-	-	0.37	0.21
L11	0.21		0.04	0.03	0.10	-1.19	-1.34
L12	0.24		0.13	0.007	0.07	-0.94	-1.09
L15	0.28	(c) 0.13		0.01	0.05	-0.57	-0.72
L17	0.33		0.11	0.02	0.10	-0.21	-0.36
L18	0.33		0.12	0.01	0.04	-0.21	-0.36
L21	0.42	26.00		0.10	0.10	0.31	0.16
L23	0.79	(c) 0.26		0.05	0.10	1.69	1.53
L24	0.59	30.00		0.08	0.10	1.05	0.90
L25	0.35		0.16	<0.01	0.10	-0.08	-0.23
L26	0.34	42.11		-	-	-0.14	-0.30
L27	0.22		0.08	-	-	-1.09	-1.24
L28	0.39	(c) 0.06		0.01	0.09	0.15	0.00
L29	0.29	(c) (f) 0.02		0.03	0.10	-0.49	-0.64
L31	0.30		(a) 0.20	0.001	0.02	-0.42	-0.57
L32	0.51	(a)(b)(c) 0.17		0.025	0.10	0.74	0.58
L34	0.27		0.03	0.01	0.03	-0.65	-0.80
L37	0.28	42.57		-	0.10	-0.57	-0.72
L39	0.35	23.00		0.03	0.10	-0.08	-0.23
L41	0.32	(b) (c) 0.04		0.02	0.04	-0.28	-0.43
L43	0.45	-	-	0.05	0.10	0.46	0.31
L44	0.42	50.00		0.04	0.09	0.31	0.16
L50	0.38	(b) (c) 0.11		0.02	0.10	0.10	-0.06
L54	0.34		(f) 0.02	0.005	0.01	-0.14	-0.30
L56	0.52		0.09	<0.10	0.10	0.79	0.63
L57	0.40	(c) (f) 0.03		0.04	0.10	0.21	0.05
L60	0.23	(c) 0.07		0.01	0.10	-0.99	-1.15
L63	0.50	26.00		-	-	0.69	0.54
L65	0.39		0.08	0.01	0.10	0.15	0.00
L66	1.78	-	-	-	-	3.45	3.30
L67	<2.13	-	-	-	0.39	*	*
L68	0.40		0.07	0.01	0.35	0.21	0.05
L69	0.22		(a) 0.05	0.05	0.10	-1.09	-1.24
L71	0.35		(a) 0.11	0.01	0.10	-0.08	-0.23
L72	0.29		0.10	0.01	0.06	-0.49	-0.64
L75	0.31		(a) 0.11	-	-	-0.35	-0.50
L78	0.34	27.15		-	-	-0.14	-0.30
L86	<LOD	-	-	-	-	*	*

Table 9. z-scores for oilseed rape event GT73 genomic DNA level 1 (L1) for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean is reported for information purpose only, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (f) *U* seems to be underestimated, (g) reported in m/m %, (h) seems to be reported in absolute copy numbers. Results are as submitted by participants.

Oilseed event GT73							
	Robust mean = *1.11 cp/cp %*						
Laboratory	Assigned value = 0.39 cp/cp %						
number	**Value**	**Uncertainty**		**LOD cp/cp**	**LOQ cp/cp**	**z-score[1]**	**z-score[2]**
		relative	**absolute**				
L01	**0.65**	(b) (f) 2.13		0.02	0.04	***-1.16***	**1.12**
L07	**1.71**	20.10		(h) 10.00	-	***0.94***	**3.23**
L08	**1.32**	-	-	(g) 0.10	(g) 0.10	***0.38***	**2.66**
L13	**1.34**	-	-	-	-	***0.41***	**2.70**
L14	**4.52**	(f) 6.10		0.01	0.05	***3.05***	**5.34**
L16	**1.37**		(a) (f) 0.26	<0.05	0.20	***0.46***	**2.74**
L19	**0.94**		(a) 0.63	-	0.10	***-0.36***	**1.93**
L20	**1.00**	(b) 20.00		0.05	0.10	***-0.22***	**2.06**
L30	**0.92**	(b) 31.00		-	-	***-0.40***	**1.88**
L36	**0.94**		0.30	0.10	0.20	***-0.36***	**1.93**
L40	**8.20**		(d) 18.56	-	-	***4.35***	**6.63**
L45	**1.54**	(c) 0.49		0.05	0.10	***0.72***	**3.00**
L46	**0.90**		0.40	-	-	***-0.45***	**1.83**
L47	**0.60**		0.52	-	0.10	***-1.33***	**0.95**
L51	**0.78**		(a) (f) 0.04	-	0.10	***-0.76***	**1.52**
L53	**1.22**		(f) 0.09	(h) 6.00	(h) 68.00	***0.21***	**2.49**
L55	**1.20**		0.33	0.01	0.04	***0.18***	**2.46**
L58	**1.29**	(a) 17.80		0.04	0.09	***0.33***	**2.61**
L59	**0.76**		0.10	0.05	0.10	***-0.82***	**1.46**
L61	**0.98**	27.36		0.02	0.18	***-0.27***	**2.01**
L62	**1.99**	-	-	-	-	***1.27***	**3.55**
L73	**>0.05**	-	-	0.05	-	*****	*****
L74	**1.30**	-	-	(g) 0.10	(g) 0.10	***0.35***	**2.63**
L76	**0.58**	(c) 0.40		0.10	0.10	***-1.40***	**0.88**
L77	**0.70**		0.28	-	-	***-1.00***	**1.29**
L79	**1.24**	15.74		0.01	0.01	***0.25***	**2.53**
L81	**0.92**		0.25	0.04	0.08	***-0.40***	**1.88**
L83	**0.99**	(c) 0.26		0.10	0.10	***-0.24***	**2.04**
L84	**1.34**	-	-	(g) 0.05	(g) 0.10	***0.41***	**2.70**
L85	**1.44**		1.10	0.11	0.32	***0.57***	**2.85**

Table 10. z-scores for oilseed rape event GT73 genomic DNA level 2 (L2) for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, [1] z-score calculated on the basis of the robust mean is reported for information purpose only, [2] z-score calculated on the basis of the assigned value, * = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (f) *U* seems to be underestimated, (g) reported in m/m %, (h) seems to be reported in absolute copy numbers. Results are as submitted by participants.

Oilseed event GT73 Robust mean = *0.39 cp/cp %* Assigned value = 0.15 cp/cp %							
Laboratory number	**Value**	**Uncertainty**		**LOD cp/cp**	**LOQ cp/cp**	**z-score[1]**	**z-score[2]**
		relative	**absolute**				
L85	**0.43**		0.30	0.11	0.32	***0.24***	**2.26**
L84	**0.56**	-	-	(g) 0.05	(g) 0.10	***0.81***	**2.84**
L83	**0.38**	(c) 0.26		0.10	0.10	***-0.03***	**2.00**
L81	**0.29**		0.10	0.04	0.08	***-0.62***	**1.41**
L79	**0.45**	17.90		0.01	0.01	***0.34***	**2.36**
L77	**0.20**		0.10	-	-	***-1.42***	**0.60**
L76	**0.19**	(c) 0.13		0.10	0.10	***-1.53***	**0.49**
L74	**0.43**	-	-	(g) 0.10	(g) 0.10	***0.24***	**2.26**
L73	**>0.05**	-	-	0.05	-	*****	*****
L62	**0.73**	-	-	-	-	***1.39***	**3.41**
L61	**0.32**	39.60		0.02	0.18	***-0.42***	**1.60**
L59	**0.23**		0.03	0.05	0.10	***-1.12***	**0.91**
L58	**0.40**	(a) 17.80		0.04	0.09	***0.08***	**2.11**
L55	**0.40**		0.12	0.01	0.04	***0.08***	**2.11**
L53	**0.51**		(f) 0.04	(h) 6.00	(h) 68.00	***0.61***	**2.63**
L51	**0.23**		(a) 0.05	-	0.10	***-1.12***	**0.91**
L47	**0.23**		0.20	-	0.10	***-1.12***	**0.91**
L46	**0.31**		0.16	-	-	***-0.47***	**1.55**
L45	**0.72**	21.10		0.05	0.10	***1.36***	**3.38**
L40	**3.41**		(d) 27.69	-	-	***4.74***	**6.76**
L36	**0.37**		0.10	0.10	0.20	***-0.09***	**1.94**
L30	**0.35**	(b) 31.00		-	-	***-0.21***	**1.82**
L20	**0.40**	(b) 20.00		0.05	0.10	***0.08***	**2.11**
L19	**0.20**		(a) 0.15	-	0.10	***-1.42***	**0.60**
L16	**0.48**		(a) 0.12	<0.05	0.20	***0.48***	**2.50**
L14	**1.77**	18.01		0.01	0.05	***3.31***	**5.34**
L13	**0.55**	-	-	-	-	***0.77***	**2.80**
L08	**0.44**	-	-	(g) 0.10	(g) 0.10	***0.29***	**2.31**
L07	**0.65**	17.80		(h) 10.00	-	***1.14***	**3.16**
L01	**0.19**	(b) 12.77		0.02	0.04	***-1.53***	**0.49**

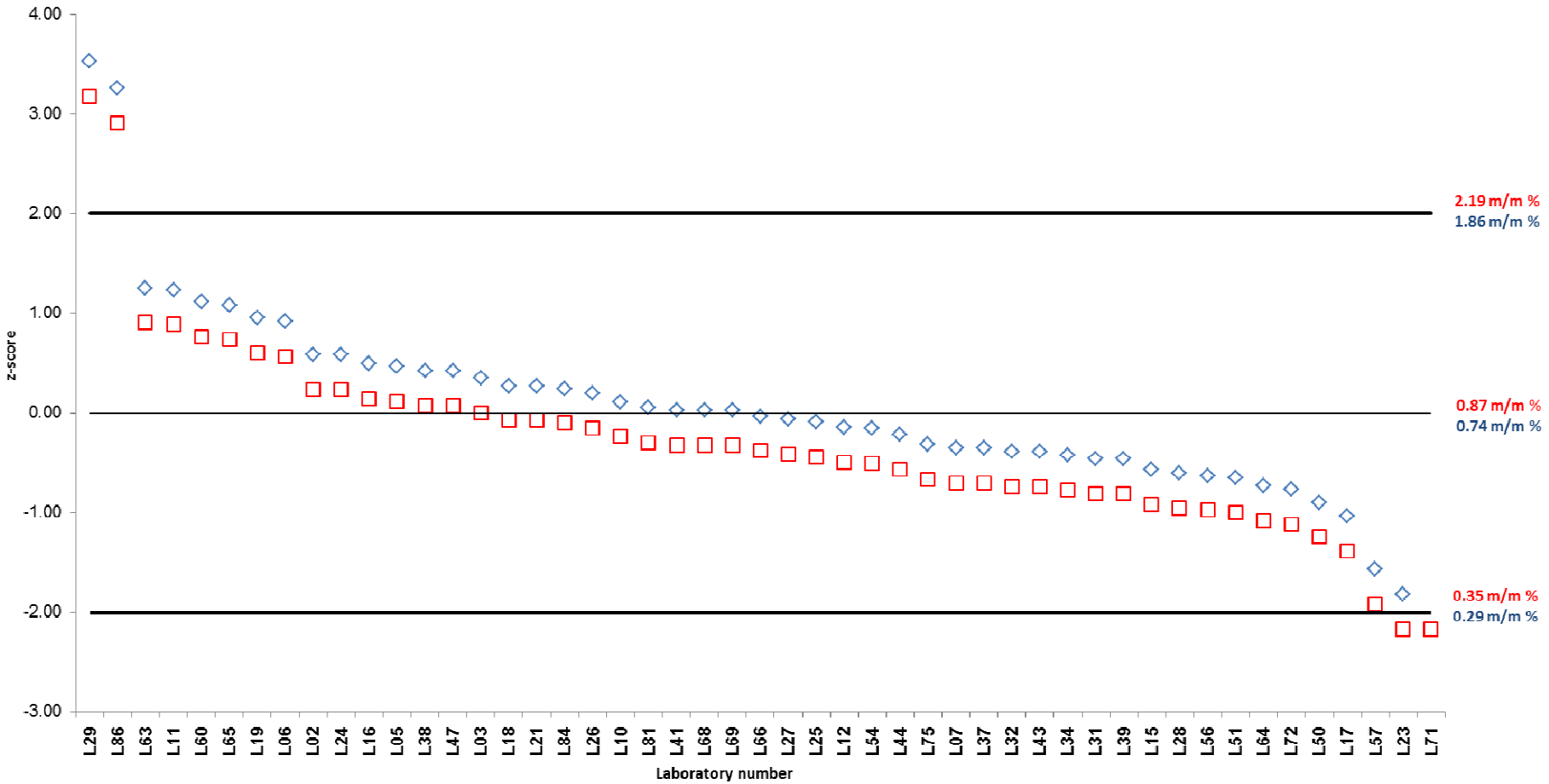

Figure 5. z-scores for maize event 59122 genomic DNA level 1 on the basis of an assigned value of 0.87 m/m % (□) and a robust mean of 0.74 m/m % (◊).

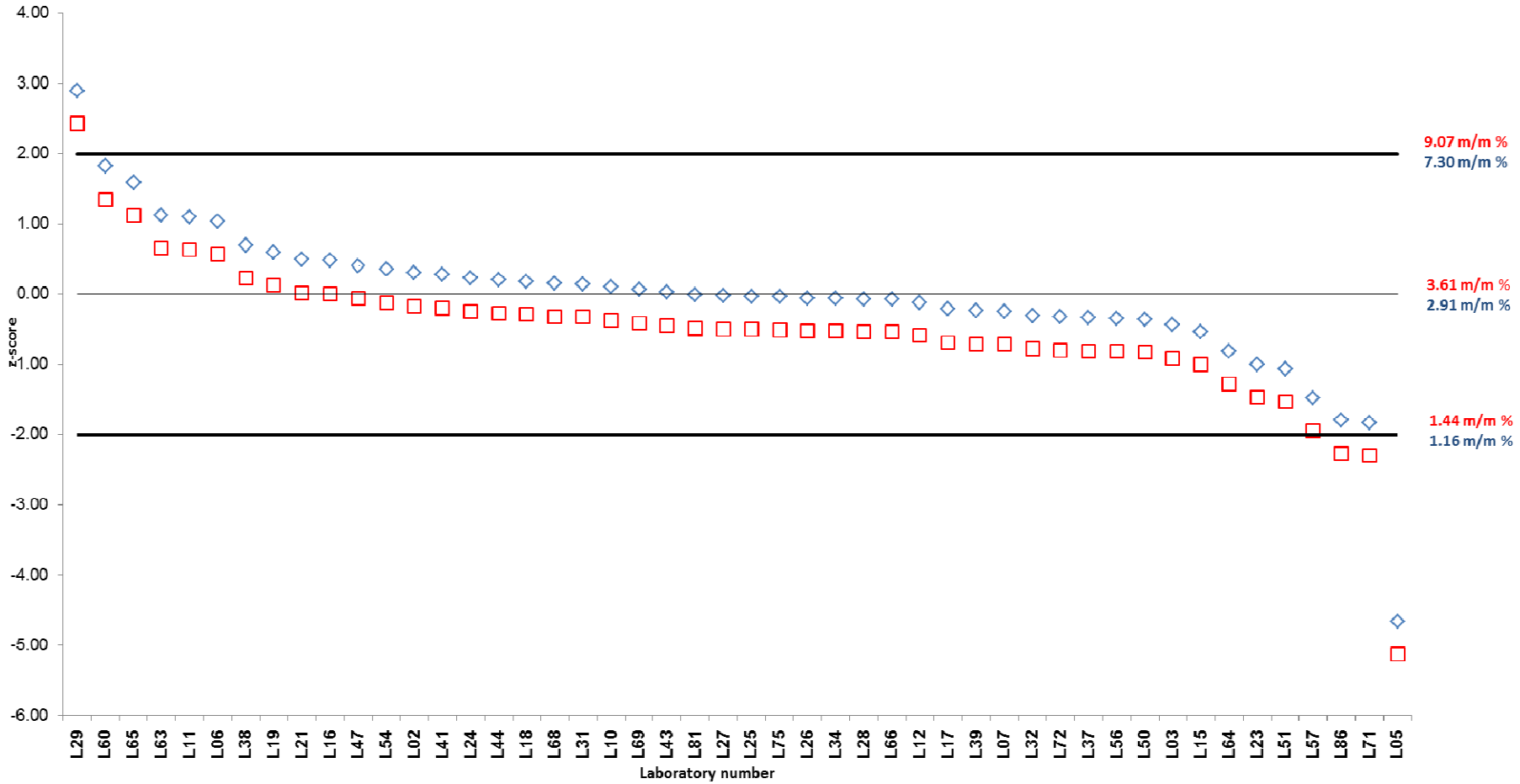

Figure 6. z-scores for maize event 59122 genomic DNA level 2 on the basis of an assigned value of 3.61 m/m % (□) and a robust mean of 2.91 m/m % (◊).

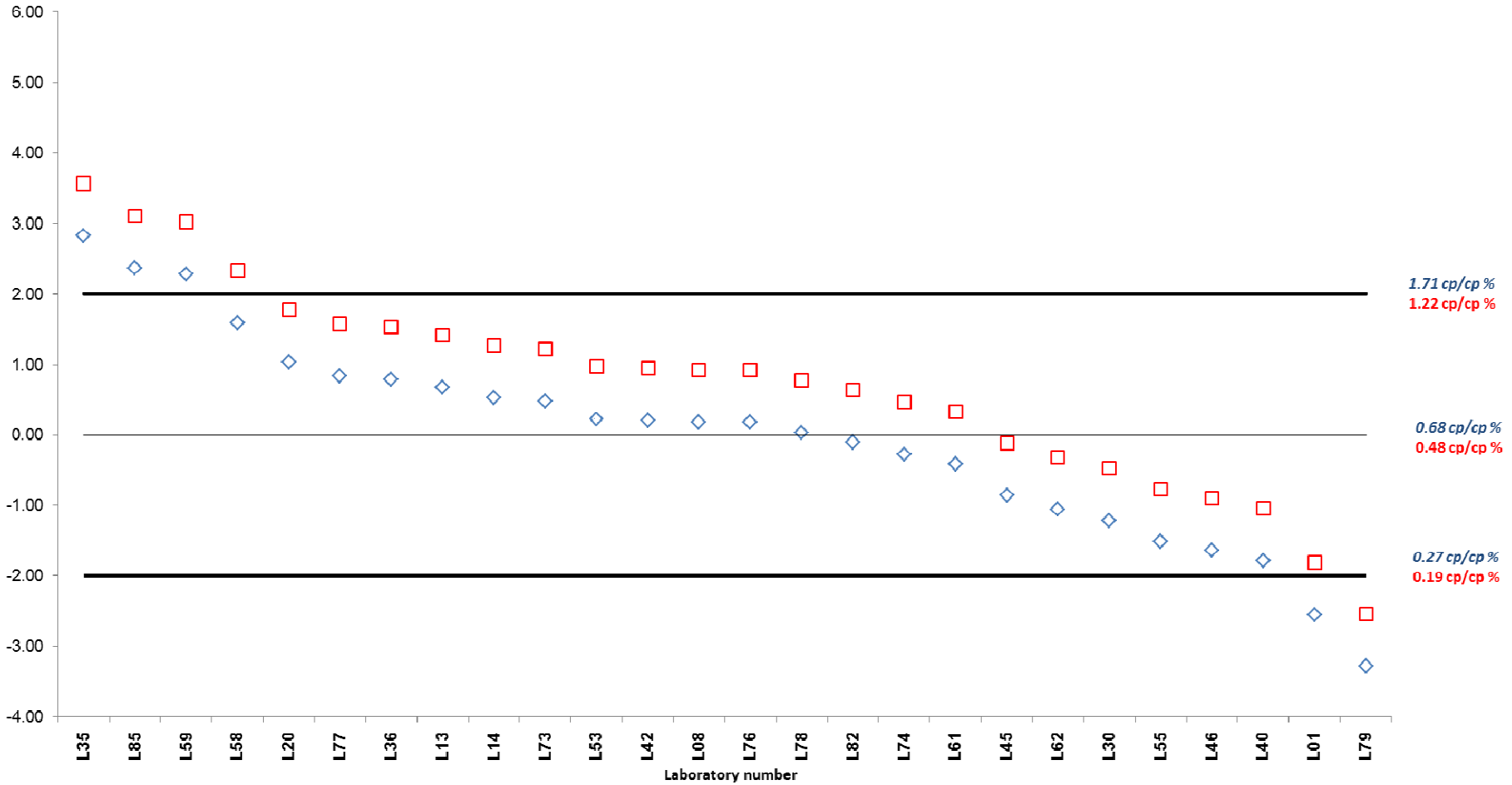

Figure 7. z-scores for maize event 59122 genomic DNA level 1 on the basis of an assigned value of 0.48 cp/cp % (□) and a robust mean of 0.68 cp/cp % (◊). The z-scores calculated on the basis of the robust mean are shown for information purpose only.

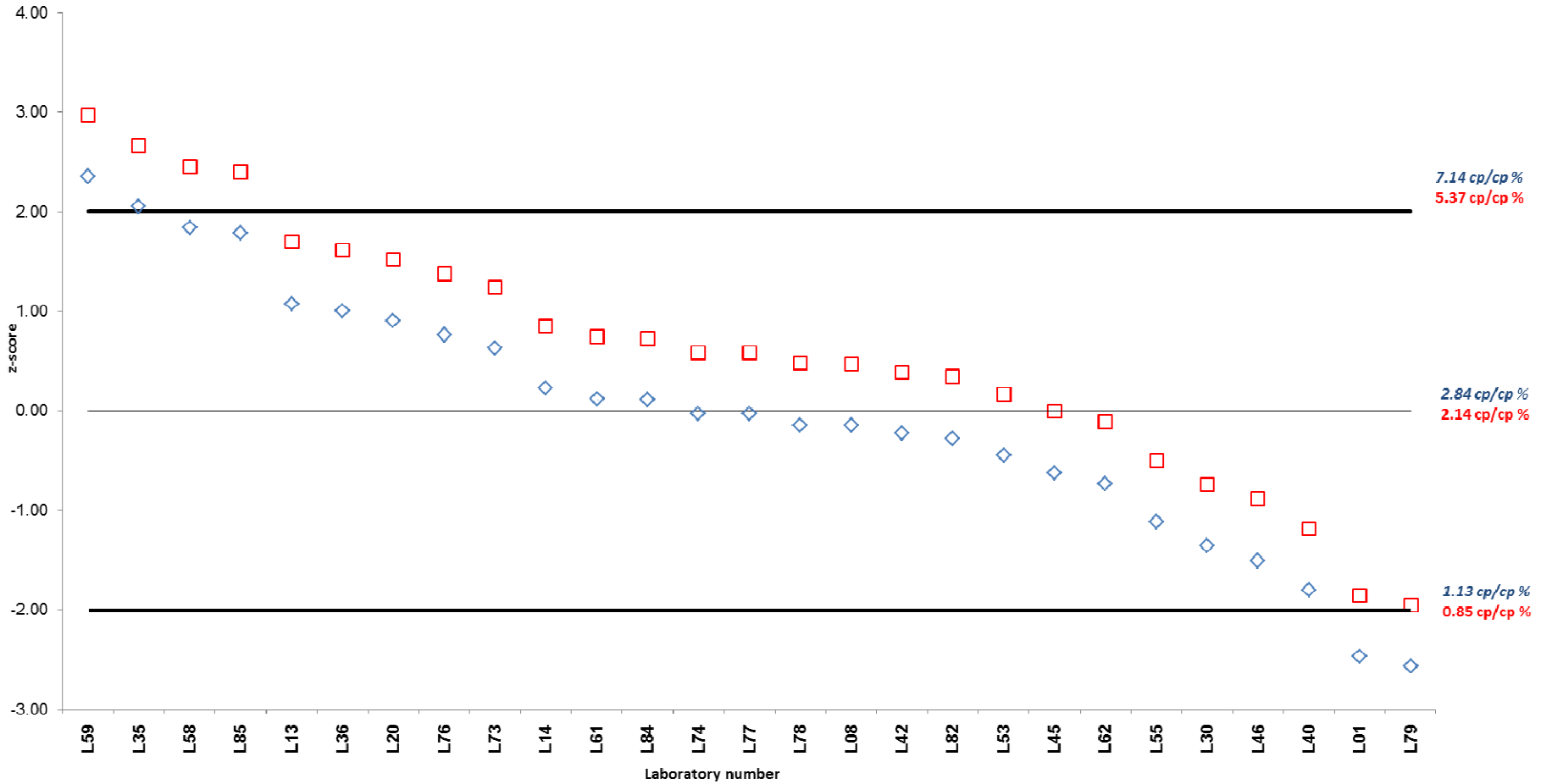

Figure 8. z-scores for maize event 59122 genomic DNA level 2 on the basis of an assigned value of 2.14 cp/cp % (□) and a robust mean of 2.84 cp/cp % (◊).The z-scores calculated on the basis of the robust mean are shown for information purpose only.

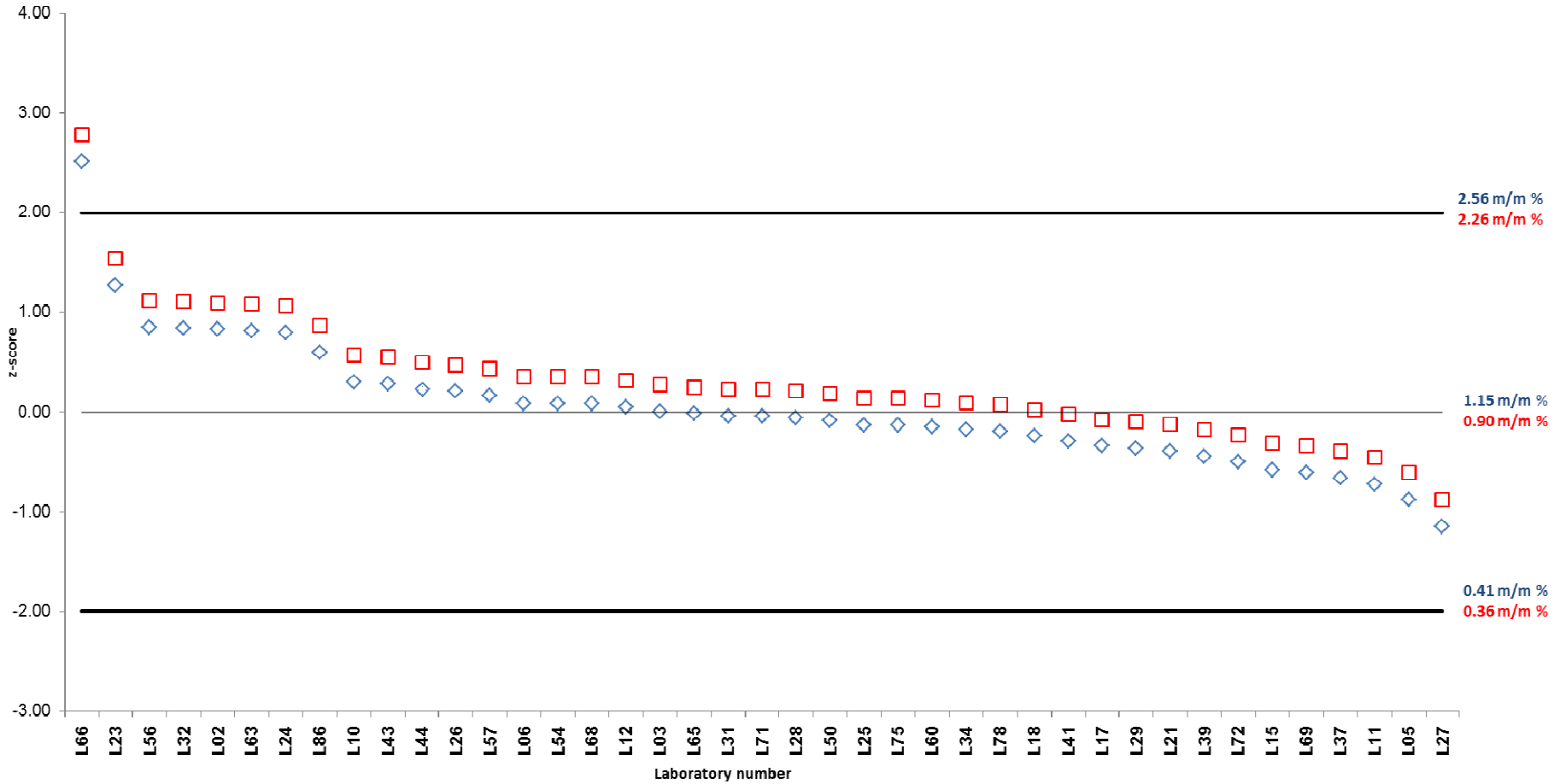

Figure 9. z-scores for oilseed rape event GT73 genomic DNA level 1 on the basis of an assigned value of 0.90 m/m % (□) and a robust mean of 1.15 m/m % (◊).

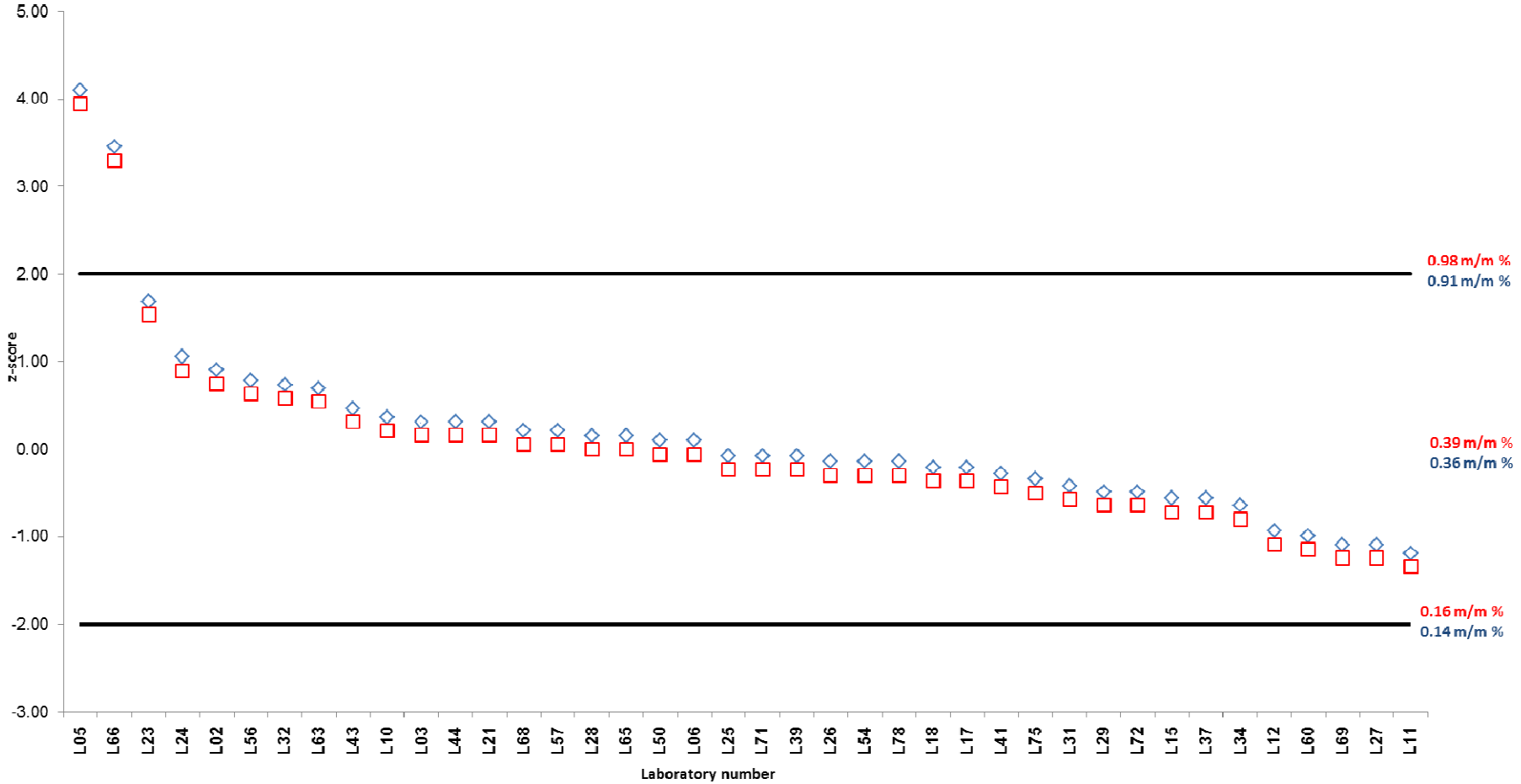

Figure 10. z-scores for oilseed rape event GT73 genomic DNA level 2 on the basis of an assigned value of 0.39 m/m % (□) and a robust mean of 0.36 m/m % (◊).

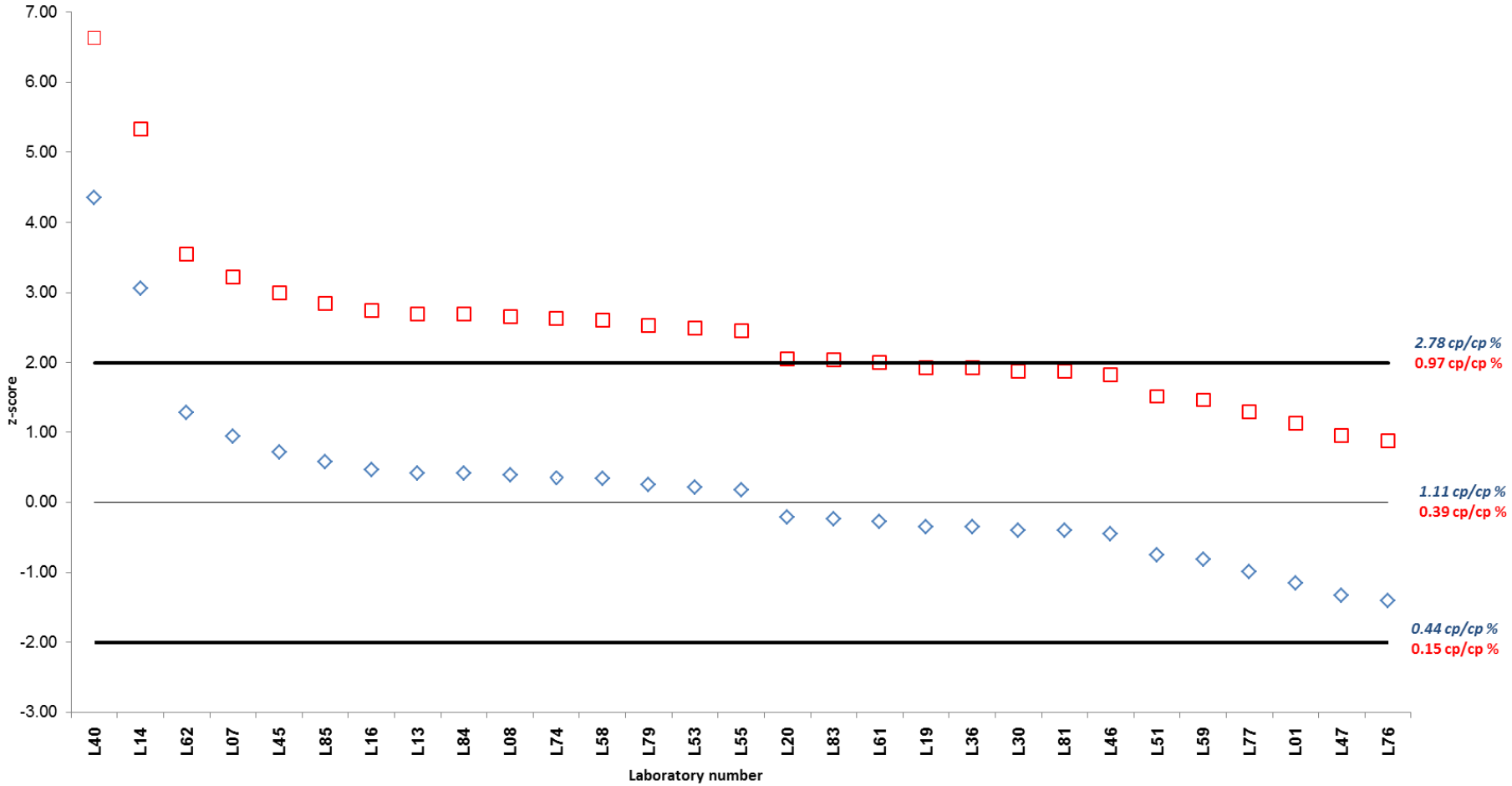

Figure 11. z-scores for oilseed rape event GT73 genomic DNA level 1 on the basis of an assigned value of 0.39 cp/cp % (□) and a robust mean of 1.11 cp/cp % (◊). The z-scores calculated on the basis of the robust mean are shown for information purpose only.

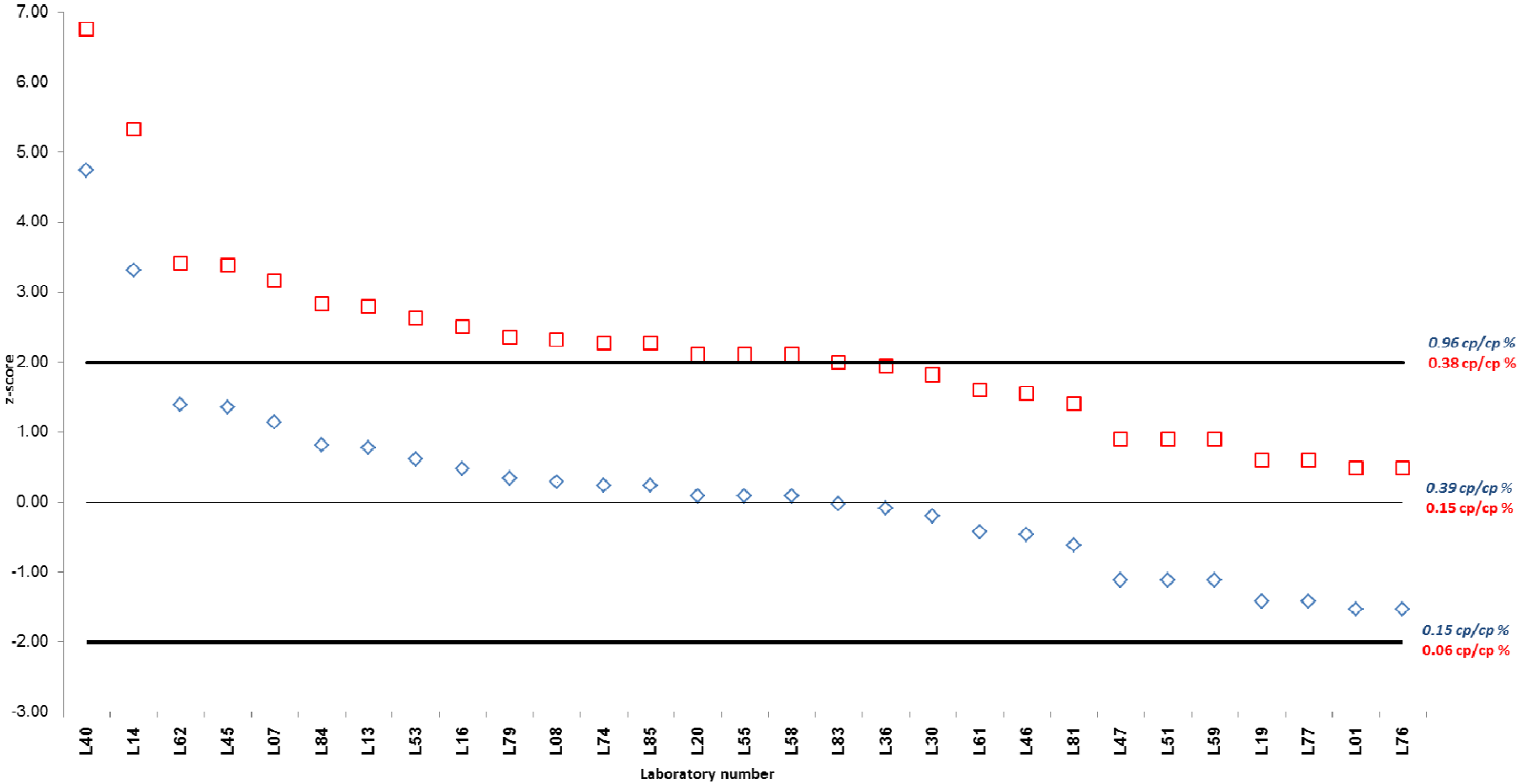

Figure 12. z-scores for oilseed rape event GT73 genomic DNA level 2 on the basis of an assigned value of 0.15 cp/cp % (□) and a robust mean of 0.39 cp/cp % (◊).The z-scores calculated on the basis of the robust mean are shown for information purpose only.

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 target standard deviation for proficiency assessment $\hat{\sigma}$ the more likely participants with a z-score outside the working range of -2 to +2 are underperforming. However a greater $\hat{\sigma}$ 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

In this fifth comparative testing round 92 to 98 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in m/m % depending on the DNA level and the GM event. However, a lower percentage (38 – 93 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in cp/cp %. The assigned values derived from the homogeneity study conducted at the EU-RL GMFF were close to the robust means expressed in m/m % (Figure 13a). There was however a disparity between the assigned values obtained through digital PCR and the robust means expressed in cp/cp % (Figure 13b).

With respect to the quantification of maize event 59122 the majority of participants that had expressed the results in cp/cp % and gained a z-score outside the range of -2 to +2, used a genomic DNA calibrant for calibration and prepared the dilution series on the basis of DNA copy numbers. It is however recommended that participants express their measurements results in m/m % when a CRM, certified for the mass fraction is used as calibrant. If users intend to use these CRMs for GM measurement results expressed in copy number ratios, they should take account of the zygosity stated in the certification report[19] and should closely follow IRMM's guidelines for the conversion of mass fraction to DNA copy number ratio[20].

With respect to the quantification of oilseed rape event GT73 nearly all laboratories that had expressed the results in cp/cp % used a genomic DNA calibrant for calibration. The CRM producer communicated that the GT73 canola seeds are homozygous. Hence the majority of laboratories using genomic DNA calibration assumed the zygosity ratio of GM to reference target to be equal to 1. However *Brassica napus* is an amphidiploid species (AACC)

possessing two genomes A and C. The DNA sequence of the reference system (*Cru A, fat A)* is present in both genomes, whereas the homozygous GM trait is only present in one of both genomes. Consequently, the zygosity ratio is expected to be 0.5 and not 1 as assumed by the majority of laboratories. Participants having expressed the results of GM quantification of oilseed rape in cp/cp % should take into account the ploidy level of the *Brassica* species quantified and should have divided their results by a factor of two.

As a consequence the robust means (μ_R) and assigned values (μ) expressed in cp/cp % are quite different. The z-scores calculated on the basis of the robust means in cp/cp % are given for information purpose only (Tables 5, 6, 9 and 10).

a)

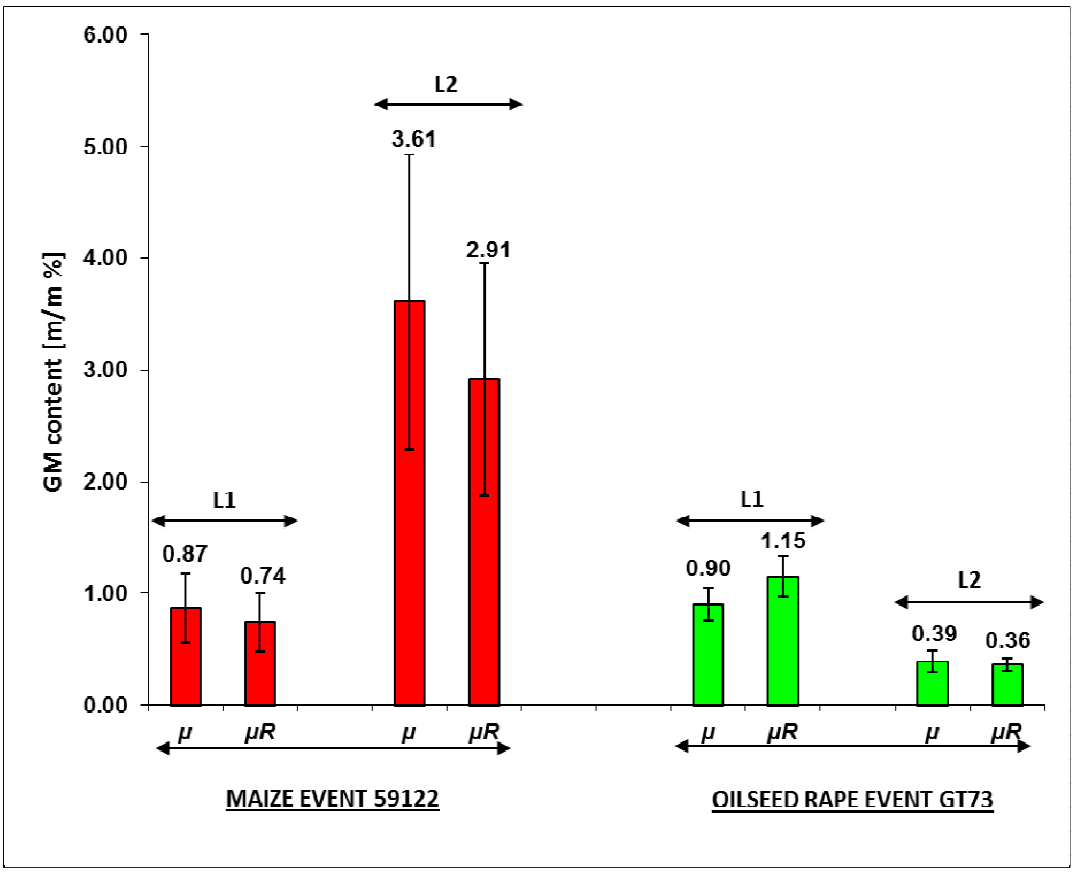

Figure 13. Comparison of assigned values (μ) and robust means (μ_R) of the genomic DNA levels 1 (L1) and 2 (L2) test items in mass/mass % (a) and in copy/copy % (b). The error bars represent the expanded uncertainties.

b)

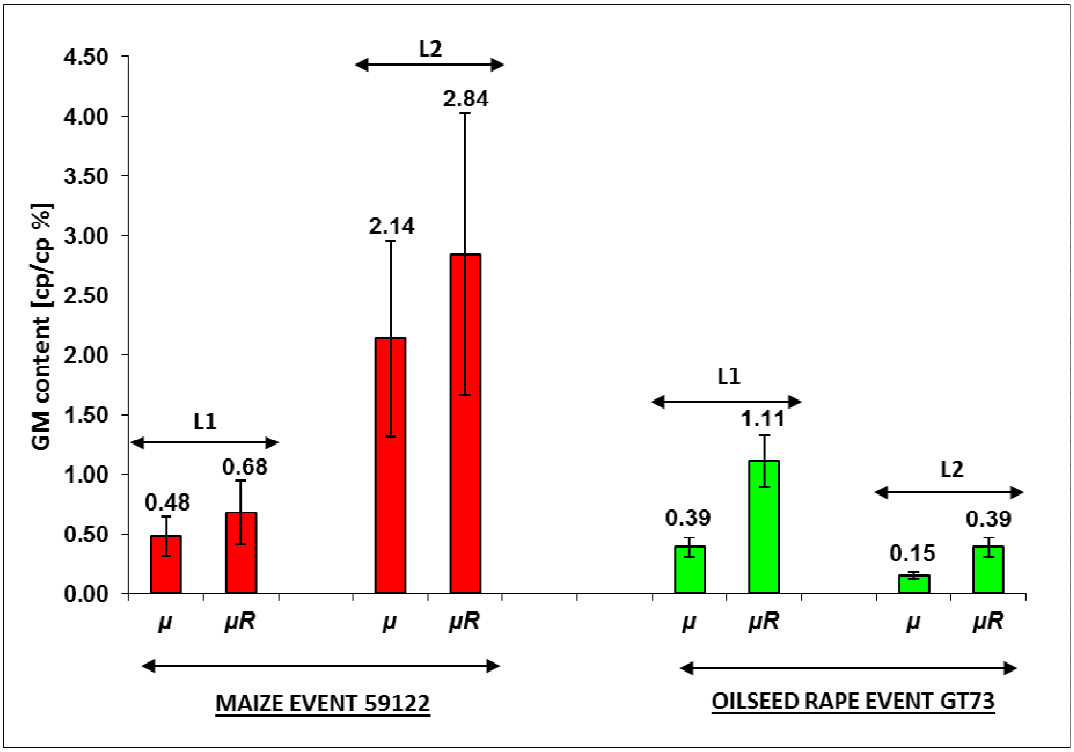

Figure 13 (continued). Comparison of assigned values (μ) and robust means (μ_R) of the genomic DNA levels 1 (L1) and 2 (L2) test items in mass/mass % (a) and in copy/copy % (b). The error bars represent the expanded uncertainties.

An overview of the laboratories having obtained outlying z-scores is provided in Table 11.

Table 11. Overview of laboratories with outlying z-scores on the basis of the assigned value for the genomic DNA levels 1 and 2 test items in mass/mass % (a) and in copy/copy % (b). - = no results reported, * = no z-score attributed .

a)

	Outlying z-scores [m/m %]			
Laboratory	Maize 59122		Oilseed rape GT73	
number	Level 1	Level 2	Level 1	Level 2
L05		x		x
L23	x			
L29	x	x		
L66			x	x
L71	x	x		
L86	x	x		*

Table 11 (continued). Overview of laboratories with outlying z-scores on the basis of the assigned value for the genomic DNA levels 1 and 2 test items in mass/mass % (a) and in copy/copy % (b).
- = no results reported, * = no z-score attributed.

b)

	Outlying z-scores [cp/cp %]			
Laboratory	Maize 59122		Oilseed rape GT73	
number	Level 1	Level 2	Level 1	Level 2
L07			x	x
L08			x	x
L13			x	x
L14			x	x
L16			x	x
L20			x	x
L35	x	x	-	-
L40			x	x
L45			x	x
L53			x	x
L55			x	x
L58	x	x	x	x
L59	x	x		
L61			x	
L62			x	x
L74			x	x
L79	x		x	x
L83	-	-	x	
L84			x	x
L85	x	x	x	x

A higher proportion of laboratories obtained a z-score outside the range of -2 to +2 for the results expressed in cp/cp %. The cause for the outlying z-scores was investigated and is summarised in Table 12.

Table 12. Overview of the possible reasons for outlying z-scores. Ct value = cycle threshold value, R^2 = coefficient of determination, NTC = no template control.

Laboratory number	Technical problems	Ct values outside working range	R^2 outside range	Slope outside range	Great DNA amount analysed	Swapped results	Possible reporting error	Possible calculation mistake	No quantification of endogenous target	Possible contamination issue
L01		X								
L05						X				
L07				X				X		
L08							X			
L13							X			
L14								X		
L16								X		
L20				X				X		
L23		X								
L29	X									
L35	X			X						
L40								X		
L45								X		
L53								X		
L55								X		
L58		X						X		
L59								X		
L61								X		
L62								X		
L66					X					X
L74							X			
L79									X	
L83								X		
L84								X		
L85			X							
L86	X					X				

In this section the terms used in Table 12 are further explained.

- 'Technical problems' may refer to problems encountered with the real-time PCR equipment or with the consumables.
- 'Ct values outside working range' means that the Ct values of the unknown samples fell beyond the linear working range of the calibration curve. Since it is not known if the calibration curve shows a linear pattern beyond its working range, it is unacceptable to extrapolate the quantification of unknown samples beyond the working range of the calibration curve.
- 'R^2 outside range' implies that the coefficient of determination (R^2) was poor compared to the acceptable value ($R^2 \geq 0.98$) outlined in the ENGL guidance document[21].
- 'Slope outside range' indicates that the slope of the calibration curve was poor compared to the acceptable values ($-3.6 \leq \text{slope} \leq -3.1$) outlined in the ENGL guidance[21].
- 'Great DNA amount analysed' means that, in all probability, the participant used a sample intake above 200 ng for a reaction volume of 50 µL in real-time PCR. The Advisory Board for comparative testing recommends that such great sample intakes should be avoided because it may reduce PCR efficiency and therefore could cause an underestimation of the actual GM content.
- 'Swapped results' either means that the participant has swapped the results reported for the genomic DNA levels 1 and 2 test items or that the results reported for maize event 59122 and oilseed rape GT73 have been swapped.
- 'Possible reporting error' indicates that those particpants should have reported their results in m/m % instead of cp/cp %.
- 'Possible calculation mistake' either indicates that IRMM's guidelines[20] for the conversion of m/m % to cp/cp % were not followed or that no account was taken of the ploidy level of *Brassica napus*.
- 'No quantification of endogenous target' means that the endogenous target was not quantified by real-time PCR.
- 'Possible contamination issue' means that amplification was noted for the negative control.

8. Performance of laboratories

Given the legal mandate of the EU-RL GMFF to organise comparative testing for NRLs and ensure an appropriate follow-up of their performance, section 8.1 focuses on the performance of NRLs. However, the performance of other participants is also monitored and they also receive suggestions to improve their performance when needed (section 8.2).

8.1 NRLs

Seventy NRLs were invited to participate in this comparative testing round. Ten NRLs did not register for this comparative testing round. One (L80) out of 60 NRLs that registered for the fifth comparative testing round did not report results. Due to a quality problem with the

primers and probes L04 could not submit results within the deadline. They received a new set of primers and probes from the company and submitted results within the deadline for the repetition of the experimental work (Tables 15a-16a).

Twelve (L05, L07, L13, L14, L16, L53, L58, L59, L61, L79, L85 and L86) out of 59 NRLs, obtained z-scores outside the working range of -2 to +2. Ten (L07, L13, L14, L16, L53, L58, L59, L61, L79, and L85) of those laboratories had expressed the results in cp/cp %. Two laboratories (L05 and L86) had expressed the results in m/m %. These z-scores were outside the range of -2 to +2 when calculated on the basis of the assigned values derived from the homogeneity study (m/m % data) and digital PCR (cp/cp % data, Tables 3 - 10). One NRL (L01) that expressed the results in cp/cp % obtained outlying z-scores for maize event 59122 calculated on the basis of the robust mean. Since the z-scores (-1.82 and -1.86) calculated on the basis of the assigned value were also close to the limit of -2, L01 was asked to submit its raw data. Analysing the raw data of these participants allowed the identification of possible causes for these results. When the outlying z-score was due to a calculation or reporting error, the participants were not asked to repeat the experimental work. L05 had swapped the results reported for maize event 59122 and oilseed rape event GT73. The exchange of the reported values led to z-scores within the range of -2 to -2. When performing real-time PCR experiments L05 is recommended to use at least two PCR replicates. The use of only one PCR replicate might lead to a bias in the results. Upon request of further information L13 communicated that the results should have been reported in m/m % instead of cp/cp %. Shifting the results to the m/m % data resulted in z-scores within the range of -2 to +2. L14, L16, L53, L58 and L61 did not take account of the ploidy level of *Brassica napus*. With the exception of L14 division of the reported values by two resulted in z-scores within the range of -2 to +2. With respect to the quantification of maize event 59122, L58 used a CRM certified for the mass fraction from IRMM but prepared the calibration curve in DNA copy numbers. The Ct values of the positive control sample fell beyond the linear working range of the calibration curve. L58 is recommended to take account of IRMM's guidelines when using their CRMs and to use a TE buffer with a lower concentration in EDTA (e.g. TE 0.1X: 10 mM Tris-HCl, 0.1 mM EDTA or TE low: 1 mM Tris, 0.01 mM EDTA) compared to the standard TE buffer. The use of a high EDTA concentration is disadvised because it might inhibit the PCR reaction. One NRL (L07) that did not take account of the ploidy level of *Brassica napus* (i.e. 'calculation error') was asked to repeat the experimental work. L07 obtained poor slopes, for both the endogenous and GM target calibration curves of the oilseed rape GT73 event, compared to the acceptable values ($-3.6 \leq$ slope ≤ -3.1) outlined in the ENGL guidance[21]. Moreover the material used for calibration was poorly characterised. L07 used DNA extracted from leaves for calibration and expressed the results in cp/cp % without having any knowledge about the zygosity status of the leaf material. L59 and L85 have been requested to submit their raw data but these data were not received. Upon submission of their results L86 reported a mistake (i.e. single base change) in the probe sequence used for the quantification of oilseed rape event GT73. The reported Limit of Quantification (LOQ) is in contradiction with the value for the genomic DNA level 1 test item reported for oilseed rape event GT73. Although the z-score falls in the working range of -2 to +2, the reported mistake in the probe sequence and the values for the LOD and LOQ raise doubts regarding the validity of the z-score. In addition, the values reported for maize event 59122 seem to have been swapped but this observation was not confirmed

by L86. Moreover, the reported uncertainties for maize GM event 59122 are abnormally high. One NRL (L79) did not quantify the endogenous target by real-time PCR but used the DNA copy number values reported by the EU-RL GMFF in its letter accompanying the test items. The values reported by the EU-RL GMFF were merely indicative and were intended as an aid to estimate the sample intake for real-time PCR. In the case of L01 the Ct values of the genomic DNA level 1 test item fell beyond the linear working range of the calibration curve. There was a systematic underestimation of the GM content by a factor of about 2 on the basis of the assigned value.

Six NRLs (L01, L07, L59, L79, L85, and L86) were asked to repeat the experimental work related to this fifth comparative testing round. L04 had not yet submitted any results but the participant was asked to submit the results to allow the EU-RL GMFF reporting these results in the section 'Results of the repetition of 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. The advice was in line with the observations noted in Table 12 for each participant.

8.2 Non-NRLs

Thirteen (L08, L20, L23, L29, L35, L40, L45, L55, L62, L66, L71, L74, L83 and L84) out of 21 non-NRLs, obtained z-scores outside the working range of -2 to +2. One non-NRL (L08, L74) registered twice and submitted both sets of results in cp/cp %. Nine (L08, L20, L35, L40, L45, L55, L62, L74, L83 and L84) of those laboratories had expressed the results in cp/cp %. Four laboratories (L23, L29, L66 and L71) had expressed the results in m/m %. Analysing the raw data of those participants allowed identifying possible causes for these results. When the outlying z-score was due to a calculation or reporting error, the participants were not asked to repeat the experimental work. L08, L40, L45, L55, L58, L62, L74, L83 and L84 did not take account of the ploidy level of *Brassica napus*. With the exception of L40 and L62 division of the reported values by two resulted in z-scores within the range of -2 to +2. L58 also obtained z-scores outside the working range of -2 to +2 for maize event 59122. This participant used a genomic DNA calibrant from IRMM for calibration and prepared the dilution series on the basis of DNA copy numbers. It is however recommended that participants express their measurements results in m/m % when a CRM, certified for the mass fraction is used as calibrant. They should take account of the zygosity stated in the certification report[19] and should closely follow IRMM's guidelines for the conversion of mass fraction to DNA copy number ratio[20]. In the case of L23 the Ct values of unknown samples fell beyond the linear working range of the calibration curve. However, the quantification of unknown samples cannot be extrapolated beyond the working range of the calibration curve. L23 is recommended to use a TE buffer with a lower concentration in EDTA (e.g. TE 0.1X: 10 mM Tris-HCl, 0.1 mM EDTA or TE low: 1 mM Tris, 0.01 mM EDTA) compared to the standard TE buffer. Upon request of the raw data of L29 the participant reported problems with the calibration of the real-time PCR instrument and submitted already the results of the repeated experimental work. Even in the repeated analyses the issue with the real-time PCR equipment remained. In the multicomponent plot the TAMRA signal was constant or slightly increasing instead of decreasing. In the reference target system there is quite a large

variance of the ROX signal whereas the fluorescence should remain constant. The ROX signal in the GM target seems more stable. The raw data submitted by L66 for oilseed rape event GT73 show a very poor amplification making it impossible to distinguish between signal and noise. Since L66 did not submit any questionnaire data it was difficult to check the experimental setup of the analyses conducted. Obviously the real-time PCR method for oilseed rape event GT73 should be validated. Upon request from the EU-RL GMFF, L71 submitted its raw data but in a format incompatible with the EU-RL GMFF software. In addition, little information was given in the questionnaire. Judging from the results submitted L71 seems to have underestimated the GM content of the maize event 59122 by a factor of 2. L71 used a delta Ct method for quantification.

Six non-NRLs (L20, L23, L29, L35, L66 and L71) were asked to repeat the experimental work related to this fifth comparative testing round. Before the shipment of a new set of test items advice was provided regarding the approach to be followed for the experimental analyses. The advice was in line with the observations noted in Table 12 for each participant.

8.3 Correction of results by the EU-RL GMFF without the need for repeating the experimental work

During data analysis the EU-RL GMFF contacted a number of participants to request further clarification. A number of errors were encountered which could be solved without the need for repeating the experimental work. In the case of oilseed rape event GT73 the results of all laboratories who reported the results in cp/cp % but did not take account of the ploidy level of *Brassica napus* were divided by a factor of 2. The EU-RL GMFF corrected the submitted results taking account of the information received from the participants and recalculated the robust means and the z-scores. Since the uncertainty data might have been affected by the correction of the results, all uncertainty data were omitted. The results are summarised in the Tables below.

Table 13. Corrections for maize event 59122 genomic DNA levels 1 and 2 for results reported in m/m %. [1] corrected z-score obtained on the basis of the recalculated robust mean, [2] corrected z-score calculated on the basis of the assigned value. Results were corrected by the EU-RL GMFF according to the information received from participants.

Maize event 59122				
Laboratory number	**Recalculated robust mean = 0.74 m/m % Assigned value = 0.87 m/m %**			
Level 1	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L05	**0.92**	0.68	**-0.18**	**-0.54**
L08	**0.74**	0.74	**0.00**	**-0.35**
L13	**0.93**	0.93	**0.49**	**0.14**
L74	**0.60**	0.60	**-0.46**	**-0.81**
	Recalculated robust mean = 2.95 m/m % Assigned value = 3.61 m/m %			
Level 2	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L05	**0.34**	2.40	**-0.45**	**-0.89**
L08	**2.65**	2.65	**-0.24**	**-0.67**
L13	**4.66**	4.66	**0.99**	**0.55**
L74	**2.80**	2.80	**-0.12**	**-0.55**

In the case of maize event 59122 all corrections introduced resulted in z-scores within the working range of -2 to +2. L05 swapped the results reported for maize event 59122 and oilseed rape event GT73. L08, L13 and L74 reported that their results should have been submitted in m/m % instead of cp/cp %.

L58 used a CRM from IRMM for calibration and prepared the calibration curves in DNA copy numbers. As it is not known if L58 took into account the zygosity of maize event 59122, the values of L58 were not corrected in Table 13. Division of the values by a factor of two results in corrected z-scores of 0.33[1] and 0.83[2] for maize event 59122 level 1 and 0.57[1] and 0.95[2] for level 2 ([1, 2] see legend Table 13). It thus seems likely that L58 did not take into account the zygosity of maize event 59122.

Table 14. Corrections for oilseed rape event GT73 genomic DNA levels 1 and 2 for results reported in m/m % (a) and cp/cp % (b). [1] corrected z-score calculated on the basis of the recalculated robust mean, [2] corrected z-score calculated on the basis of the assigned value. For the results expressed in cp/cp % the z-score calculated on the basis of the robust mean is given for information purpose only. Results were corrected by the EU-RL GMFF according to the information received from participants.

a)

Oilseed rape event GT73				
Laboratory number	**Recalculated robust mean = 1.04 m/m %** **Assigned value = 0.90 m/m %**			
Level 1	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L05	0.68	**0.92**	**-0.27**	**0.05**
L08	1.32	**1.32**	**0.51**	**0.83**
L13	1.34	**1.34**	**0.55**	**0.86**
L74	1.30	**1.30**	**0.48**	**0.80**
	Recalculated robust mean = 0.37 m/m % **Assigned value = 0.39 m/m %**			
Level 2	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L05	2.40	**0.34**	**-0.17**	**-0.30**
L08	0.44	**0.44**	**0.39**	**0.26**
L13	0.55	**0.55**	**0.87**	**0.75**
L74	0.43	**0.43**	**0.34**	**0.21**

Table 14 (continued). Corrections for oilseed rape event GT73 genomic DNA levels 1 and 2 for results reported in m/m % (a) and in cp/cp % (b). [1] corrected z-score calculated on the basis of the recalculated robust mean, [2] corrected z-score calculated on the basis of the assigned value. For the results expressed in cp/cp % the z-score calculated on the basis of the robust mean is given for information purpose only. Results were corrected by the EU-RL GMFF according to the information received from participants.

b)

Oilseed rape event GT73				
Laboratory number	Recalculated robust mean = *0.61 cp/cp %* Assigned value = 0.39 cp/cp %			
Level 1	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L07	1.71	**0.86**	***0.73***	**1.72**
L14	4.52	**2.26**	***2.84***	**3.83**
L16	1.37	**0.69**	***0.24***	**1.24**
L19	0.94	**0.47**	***-0.57***	**0.42**
L20	1.00	**0.50**	***-0.44***	**0.56**
L30	0.92	**0.46**	***-0.62***	**0.37**
L40	8.20	**4.10**	***4.13***	**5.12**
L45	1.54	**0.77**	***0.50***	**1.49**
L46	0.90	**0.45**	***-0.67***	**0.33**
L47	0.60	**0.30**	***-1.55***	**-0.55**
L51	0.78	**0.39**	***-0.98***	**0.02**
L53	1.22	**0.61**	***-0.01***	**0.99**
L55	1.20	**0.60**	***-0.04***	**0.95**
L58	1.29	**0.65**	***0.11***	**1.11**
L59	0.76	**0.38**	***-1.04***	**-0.04**
L61	0.98	**0.49**	***-0.49***	**0.50**
L62	1.99	**1.00**	***1.06***	**2.05**
L76	0.58	**0.29**	***-1.62***	**-0.63**
L77	0.70	**0.35**	***-1.21***	**-0.22**
L81	0.92	**0.46**	***-0.62***	**0.37**
L83	0.99	**0.50**	***-0.46***	**0.53**
L84	1.34	**0.67**	***0.20***	**1.19**
	Recalculated robust mean = *0.20 cp/cp %* **Assigned value = 0.15 cp/cp %**			
Level 2	**Value**	**Corrected value**	**z-score[1]**	**z-score[2]**
L07	0.65	**0.33**	***1.02***	**1.66**
L14	1.77	**0.89**	***3.20***	**3.83**
L16	0.48	**0.24**	***0.36***	**1.00**
L19	0.20	**0.10**	***-1.54***	**-0.90**
L20	0.40	**0.20**	***-0.03***	**0.60**
L30	0.35	**0.18**	***-0.32***	**0.31**
L40	3.41	**1.71**	***4.62***	**5.26**
L45	0.72	**0.36**	***1.24***	**1.88**
L46	0.31	**0.16**	***-0.59***	**0.05**
L47	0.23	**0.12**	***-1.23***	**-0.60**
L51	0.23	**0.12**	***-1.23***	**-0.60**
L53	0.55	**0.26**	***0.49***	**1.13**
L55	0.40	**0.20**	***-0.03***	**0.60**
L58	0.40	**0.20**	***-0.03***	**0.60**
L59	0.23	**0.12**	***-1.23***	**-0.60**
L61	0.32	**0.16**	***-0.55***	**0.09**
L62	0.73	**0.37**	***1.27***	**1.91**
L76	0.19	**0.10**	***-1.65***	**-1.01**
L77	0.20	**0.10**	***-1.54***	**-0.90**
L81	0.29	**0.15**	***-0.73***	**-0.10**
L83	0.38	**0.19**	***-0.14***	**0.49**
L84	0.56	**0.28**	***0.70***	**1.33**

All corrections introduced for the results expressed in m/m % for oilseed rape event GT73 resulted in z-scores within the working range of -2 to +2. L05 swapped the results reported for maize event 59122 and oilseed rape event GT73. L08, L13 and L74 reported that their results should have been submitted in m/m % instead of cp/cp % (Table 14a).

After division of the submitted results for oilseed rape event GT73 in cp/cp % by a factor of 2, all z-scores were within the working range of -2 to +2 with the exception of L14, L40 and L62 (Table 14b). All the participants listed in Table 14b did not take account of the ploidy level of *Brassica napus*. The CRM producer communicated that the GT73 canola seeds are homozygous. Hence the majority of laboratories using genomic DNA calibration assumed the zygosity ratio of GM to reference target to be equal to 1. However *Brassica napus* is an amphidiploid species (AACC) possessing two genomes and the reference system (*Cru A*, *fat A)* is present in both genomes, whereas the homozygous GM trait can only be present in one of both genomes. Consequently, the zygosity ratio is expected to be 0.5 and not 1 as assumed by the majority of laboratories.

8.3.1 NRLs

With the exception of L14, all NRLs (L05, L13, L16, L53, L58 and L61) for which the EU-RL GMFF had corrected the reported values obtained z-scores within the range of -2 to +2 (Tables 13 and 14). However, it is the responsibility of the participant in a comparative testing round to submit results in a correct way. Hence reporting errors are displayed in the original Tables (i.e. Tables 3 to 10) whereas corrected values and the results of the repetition of the experimental work are shown in Tables 13 to 16.

8.3.2 Non-NRLs

With the exception of L40 and L62, all non-NRLs (L08, L20, L45, L55, L62, L74, L83 and L84) for which the EU-RL GMFF had corrected the reported values obtained z-scores within the range of -2 to +2 (Tables 13 and 14). Since it is the responsibility of the participant in a comparative testing round to submit results in a correct way, the corrected values and the results of the repetition of the experimental work are shown in Tables 13 to 16.

8.4 Results of the repetition of the experimental work

Twelve participants (L01, L07, L20, L23, L29, L35, L59, L66, L71, L79, L85 and L86) were asked to repeat the experimental work. L07 could not repeat the experimental work due to time constraints. L35 reported problems with the delivery of the test parcel.

The results of the repetition of the experimental work are depicted in Tables 15 and 16. Participants with outlying z-scores were asked to repeat the experimental work only for those GM events where z-scores outside the working range of -2 to +2 were observed.

Table 15. Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for maize event 59122 genomic DNA levels 1 and 2. [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, - = not reported. For the results expressed in cp/cp % the z-score calculated on the basis of the robust mean is given for information purpose only. Results are as submitted by participants.

a)

Maize event 59122				
Laboratory number	**Recalculated robust mean = 0.75 m/m %** **Assigned value = 0.87 m/m %**			
Level 1	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L01	**0.76**	0.14	**0.04**	**-0.29**
L04	**1.26**	-	**1.14**	**0.80**
L23	**0.61**	0.18	**-0.44**	**-0.77**
L29	**0.39**	0.32	**-1.41**	**-1.74**
L71	**0.72**	0.14	**-0.08**	**-0.41**
L79	**1.20**	0.20	**1.03**	**0.70**
L86	**2.51**	83.00%	**2.63**	**2.30**
	Recalculated robust mean = 2.92 m/m % **Assigned value = 3.61 m/m %**			
Level 2	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L01	**2.94**	0.72	**0.01**	**-0.45**
L04	**3.50**	-	**0.39**	**-0.07**
L23	**2.07**	0.52	**-0.75**	**-1.21**
L29	**1.35**	0.15	**-1.68**	**-2.14**
L71	**2.43**	0.49	**-0.40**	**-0.86**
L79	**4.30**	0.70	**0.84**	**0.38**
L86	**0.81**	71.00%	**-2.79**	**-3.25**

b)

Maize event 59122				
Laboratory number	**Recalculated robust mean = *0.61 cp/cp %*** **Assigned value = 0.48 cp/cp %**			
Level 1	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L01	**0.25**	0.04	***-1.94***	**-1.44**
L59	**2.38**	1.06	***2.95***	**3.46**
L85	**0.71**	0.16	***0.33***	**0.83**
	Recalculated robust mean = *2.55 cp/cp %* **Assigned value = 2.14 cp/cp %**			
Level 2	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L01	**1.01**	0.24	***-2.01***	**-1.63**
L59	**13.55**	2.14	***3.63***	**4.01**
L85	**2.77**	0.43	***0.18***	**0.56**

Table 16. Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for oilseed rape event GT73 genomic DNA levels 1 and 2. [1] z-score calculated on the basis of the robust mean, [2] z-score calculated on the basis of the assigned value, - = not reported. For the results expressed in cp/cp % the z-score calculated on the basis of the robust mean is given for information purpose only. Results are as submitted by participants.

a)

Oilseed rape event GT73				
Laboratory number	**Recalculated robust mean = 1.04 m/m %** **Assigned value = 0.90 m/m %**			
Level 1	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L04	**0.92**	0.14	**-0.26**	**0.05**
L20	**0.60**	28.00%	**-1.19**	**-0.88**
L66	**2.26**	-	**1.69**	**2.00**
L86	**1.81**	60.00%	**1.21**	**1.52**
	Recalculated robust mean = 0.37 m/m % **Assigned value = 0.39 m/m %**			
Level 2	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L04	**0.40**	0.062	**0.17**	**0.05**
L20	**0.30**	28.00%	**-0.45**	**-0.57**
L66	**0.77**	-	**1.60**	**1.48**
L86	**4.43**	55.00%	**5.40**	**5.28**

b)

Oilseed rape event GT73				
Laboratory number	**Recalculated robust mean = *0.59 cp/cp %*** **Assigned value = 0.39 cp/cp %**			
Level 1	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L85	**0.58**	0.43	***-0.02***	**0.88**
	Recalculated robust mean = *0.22 cp/cp %* **Assigned value = 0.15 cp/cp %**			
Level 2	**Value**	**Uncertainty**	**z-score[1]**	**z-score[2]**
L85	**0.97**	0.42	***3.26***	**4.03**

8.4.1 NRLs

With the exception of L86, all NRLs (L01 and L79) that had expressed the results in m/m % obtained satisfactory z-scores upon repetition of the experimental work (Tables 15a-16a). L86 repeated the analyses regarding maize event 59122 and oilseed rape event GT73. In the case of maize event 59122 they reported a higher Ct value for the genomic DNA level 1 sample compared to the level 2 sample. However, L86 reported a higher GM content for the level 1 sample than for the level 2 sample whereas the Ct values indicate the opposite namely a higher GM content for the level 2 sample. Both the values regarding the quantification of maize event 59122 and oilseed rape event GT73 are outside the working range of -2 to +2. L04 did not repeat the analyses but submitted the results for the first time due to a quality problem with the primers and probes.

Two (L59 and L85) out of three NRLs that had expressed the results in cp/cp % and had repeated the experimental work did not always improve their performance (Tables 15b-16b). L059 gained z-scores outside the range of -2 to +2 for maize event 59122. L85 gained

satisfactory z-scores when requantifying the GM content of maize event 59122 but obtained an outlying z-score upon requantification of the level 2 oilseed rape event GT73. The z-score of the third NRL (L01) repeating the analyses were close to the limits of satisfactory performance (-1.44 and -1.63 for oilseed rape event GT73 levels 1 and 2 test items).

8.4.2 Non-NRLs

All non-NRLs (L20, L23, L29, L66 and L71) expressed the results of the repetition of the experimental work in m/m % (Tables 15a-16a). All non-NRLs obtained z-scores within the range of -2 to +2 although the z-scores of L29 (-1.74 and 2.00 for maize event 59122 level 1 and 2 test items) and L66 (2.00 and 1.48 for oilseed rape event GT73 level 1 and 2 test items) were close to the limits of satisfactory performance.

9. Conclusions

In this fifth comparative testing round participants were asked to determine the GM content of maize event 59122 and oilseed rape event GT73 in two DNA solutions containing both GM events in different concentrations. Hence the proficiency of the DNA extraction step was not assessed in this comparative testing round. Both test items were produced by the EU-RL GMFF.

Results could be reported in either m/m % or cp/cp %. The majority of participants submitted the results in m/m %. A few participants submitted the results in cp/cp % using a plasmid DNA calibrant, and since it is not good practice to calculate the robust mean on a limited number of data ($N = 2$), all results expressed in cp/cp % were pooled irrespective of the DNA calibrant used. However, the EU-RL GMFF is aware that differences due to the nature of the calibrant used can be observed[22)].

In this fifth comparative testing round 92 % to 98 % of participants gained a satisfactory z-score in the range of -2 to +2 based on the assigned values (μ) and the robust means (μ_R) for the results expressed in m/m % depending on the GM content and the GM event. However, a lower percentage (38 – 93 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in cp/cp %. A disparity was observed between the assigned values (μ) obtained by digital PCR and the robust means (μ_R) expressed in cp/cp % (Tables 5, 6, 9 and 10). High z-scores (i.e. z-scores above 2 or below -2) calculated on the basis of the assigned value were observed in 19 % and 15 % of reported results for maize event 59122, and 62 % and 55 % of reported results for oilseed rape event GT73 for genomic DNA levels 1 and 2, respectively.

The assigned values derived from the homogeneity study conducted at the EU-RL GMFF were close to the robust means expressed in m/m % (0.87 versus 0.74 for level 1 and 3.61 versus 2.91 m/m % for level 2, Figure 13a). In case of the genomic DNA level 2 test item, the assigned value (μ = 3.61 ± 1.32 m/m %) is higher than the robust mean (μ_R = 2.91 m/m %) but the expanded uncertainty of the assigned value still comprises the robust mean. As stated

before, a disparity was observed between the assigned values obtained through digital PCR and the robust means expressed in cp/cp % (Figure 13b).

For maize event 59122 five out of 26 and four out of 27 participants that had expressed the results in cp/cp %, gained a z-score outside the range of -2 to +2 for genomic DNA levels 1 and 2 calculated on the basis of the assigned value. The expanded uncertainty of the level 1 assigned value (μ = 0.48 ± 0.16 cp/cp %) does not comprise the robust mean (μ_R = 0.68 cp/cp %) whereas that of the level 2 assigned value (μ = 2.14 ± 0.82 cp/cp %) does (μ_R = 2.84 cp/cp %). This implies that the disparity between the assigned value and the robust mean is not obvious in the case of the level 2 test item. The majority of participants with outlying z-scores used a genomic DNA calibrant for calibration and prepared the dilution series on the basis of DNA copy numbers. It is however recommended that participants express their measurement results in m/m % when a CRM, certified for the mass fraction is used as calibrant. Indeed, ERM-BF424 has been certified for its GM mass fraction and not for its GM copy number ratio[19]. If users intend to use ERM-BF424 for GM measurement results expressed in copy number ratios, they should take account of the zygosity stated in the certification report[19] and should closely follow IRMM's guidelines for the conversion of mass fraction to DNA copy number ratio according to the principles explained in ERM Application note 4[20].

For oilseed rape event GT73, 18 and 16 out of 29 participants that had expressed the results in cp/cp %, gained a z-score outside the range of -2 to +2 for genomic DNA levels 1 and 2 calculated on the basis of the assigned value. In fact, the expanded uncertainties of the assigned values (μ = 0.39 ± 0.08 cp/cp % and μ = 0.15 ± 0.03 cp/cp %) do not comprise the robust means (μ_R = 1.11 cp/cp % and (μ_R = 0.39 cp/cp %) for levels 1 and 2, respectively. In the case of oilseed rape *Brassica napus* participants should not only take account of the zygosity statement in the CRM certificate but also of the ploidy level of the species. The CRM producer communicated that the GT73 canola seeds are homozygous. Hence the majority of laboratories using genomic DNA calibration assumed the zygosity ratio of GM to reference target to be equal to 1. However *Brassica napus* is an amphidiploid species (AACC) possessing two genomes and the reference system (*Cru A*, *fat A)* is present in both genomes, whereas the homozygous GM trait can only be present in one of both genomes. Consequently, the zygosity ratio is expected to be 0.5 and not 1 as assumed by the majority of laboratories. As a consequence the robust means (μ_R) and assigned values (μ) expressed in cp/cp % are quite different. The z-scores calculated on the basis of the robust means in cp/cp % are given for information purpose only.

In this comparative testing round the EU-RL GMFF corrected participants' results when it concerned either a reporting or a calculation mistake. In the case of maize event 59122 the results of three participants (L05, L08, L13 and L74) were corrected, whereas in the case of oilseed rape GT73 the results of 25 participants (L05, L07, L08, L13, L14, L16, L19, L20, L30, L40, L45, L46, L47, L51, L53, L55, L58, L59, L61, L62, L74, L76, L77, L81, L83 and L84) were corrected. One participant submitted two sets of results in the same measurement unit (L08 and L74). For 23 out of the latter 25 participants no account had been taken of the ploidy level of *Brassica napus*. The total number of oilseed rape quantitative results expressed in cp/cp % was 29, which implies that corrections were carried out for 26 out of 29 submitted

results. With the exception of L14, L40 and L62 all participants for which the EU-RL GMFF had corrected the reported values obtained z-scores within the range of -2 to +2 (Tables 13 and 14). However, it is the responsibility of the participant in a comparative testing round to submit results in a correct way. Hence the originally reported values are displayed in Tables 3 to 10, whereas corrected values and the results of the repetition of the experimental work are shown in Tables 13 to 16.

Six NRLs (L01, L07, L59, L79, L85, and L86) and six non-NRLs (L20, L23, L29, L35, L66 and L71) were asked to repeat the experimental work related to this fifth comparative testing round. One NRL (L04) did initially not submit any results due to a quality problem with the primers and probes. They were asked to submit the results within the deadline for repetition of the experimental work to allow the EU-RL GMFF to evaluate their performance. L07 and L35 could 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. Three (L59, L85 and L86) out of five NRLs that repeated the experimental work, again obtained outlying z-scores (Tables 15 and 16). Two (L29 and L66) out of five non-NRLs that repeated the experimental work, again obtained outlying z-scores (Tables 15 and 16). Hence, the non-NRLs repeating the experimental work performed better than the NRLs repeating the experimental work. The repetition of the experimental work is intended as an aid for the participants to allow them to improve their performance.

The assigned values in cp/cp % obtained by digital PCR, were compared with those expressed in m/m % (Tables 3 – 10). As described in the ERM Application Note 4[20] the biological variability in hybrid maize may range from 33 % (in case of a hybrid derived from a male GM and a female non-GM) to 66 % (in case of a hybrid derived from a female GM and a male non-GM). In the case of oilseed rape event GT73 the ratio of cp/cp % to m/m % results is assumed to be equal to 0.5. The ratio of the assigned values in cp/cp % to those in m/m % were 55 % and 59 % for maize event 59122 and 43 % and 38 % for oilseed rape event GT73 for genomic DNA levels 1 and 2, respectively.

In this and in the previous comparative testing round a higher percentage of NRLs obtained a z-score outside the working range of -2 to +2 in comparison with the first three exercises. 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.

About 53 % of participants provided information on measurement uncertainty (MU) in a complete and consistent manner. The percentage of participants who reported the MU in a correct way did not improve compared to the previous comparative testing round. Hence there is a need to provide laboratories with guidance and training to harmonise the MU reported in the field of GMO detection.

Participants' assessment of results in relation to MU needs to be improved. This will have an impact on the enforcement of the 0.9 % threshold. Participants should use the same approach as the one described in Regulation (EU) No 619/2011[23]. In case that the reported value minus the expanded uncertainty is above 0.9 % the sample would have to be reported as containing GM.

10. References

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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 for reference materials?	**No. of laboratories**
a) ISO/CEN published method	26
b) EU-RL validated method	6
c) National reference method	3
d) International literature	5
e) In-house developed and optimised	10
f) Other	20
Other of which	answers referred to used Kits, see Question 4

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

a) Yes	56
b) No	12

2. Sample intake (in g) for DNA extraction from reference materials:	**No. of laboratories**
a) <0.1	2
b) 0.1- 0.2	48
c) >0.2	14
d) Other	6
Other of which	
Not specified	5

3. DNA extraction method/kit for reference materials?	**No. of laboratories**
a) CTAB	26
b) CTAB-derived	10
c) Biotecon	1
d) GeneScan GENESpin	3
e) Guanidine HCl with proteinase K	5
f) Machery Nagel Nucleospin	10
g) Promega Wizard	4
h) Qiagen DNeasy plant mini kit	3
i) TEPNEL kit	0
j) Proprietary method	0
k) Other	7
Other of which	
Modified DNeasy Blood & Tissue kit	1
For DAS59122 = b) CTAB-derived; CTAB + Prot K with incubation overnight	1
For GT73 CRM, extraction was a modified Dellaporta method, for 59122 extraction a modified CTAB method	1
CTAB + Prot K with incubation overnight	1
provided DNA solution	1
Different extraction methods for rape and maize: Rape: Swiss Food Manual (Wizard-Method)/EN 21571 A.4; Maize: EN 21571 A.3 (CTAB)	1
combined with Qiagen Plant Mini Kit and SDS	1

4. How was the clean-up of the DNA performed?	**No. of laboratories**
a) No DNA clean-up	39
b) Ethanol precipitation	8
c) Amersham MicroSpin S300	0

d) Promega Wizard DNA clean-up resin	9
e) Qiagen QIAQuick	4
f) Qiagen Genomic-Tip 20/G	0
g) Silica	2
h) Proprietary method	1
i) Other	7
Other of which	
Microspin	1
DNA extractor cleaning columns	1
No purification carried out	1
JetQuick DNA Purification Kit, Genomed	1
Modified Promega Wizard SV Genomic DNA purification system	1
Qiagen DNeasy Mini Plant Kit	1
Different clean-ups for rape and maize; Rape: Swiss Food Manual (Wizard-Method)/EN 21571 A.4; Maize: EN 21571 A.3 (CTAB) combined with Qiagen Plant Mini Kit (see above)	1

5. How have you quantified the DNA?	**No. of laboratories**
a) Gel	0
b) UV spectrophotometer	24
c) Nanodrop	27
d) Fluorometer	13
e) Other	1
f) Not applicable (i.e. DNA was not quantified)	9
Other of which	
Proprietary	1

6. Dilution buffer	**No. of laboratories**
a) TE (10 mM Tris-HCl, 1 mM EDTA)	10
b) TE 0.1X (10 mM Tris-HCl, 0.1 mM EDTA)	9
c) TE low (1 mM Tris, 0.01 mM EDTA)	2
d) Water	43
e) Other	13
Other of which	
10 mM Tris	1
TE 0.2X (2 mM Tris-HCl, 0.2 mM EDTA)	4
TE (10 mM TrisHCl, 0,2 mM EDTA)	1
TE 0.5X (10 mM Tris-HCl, 0.5 mM EDTA)	2
Elution Buffer from Kit	1
AE buffer from Qiagen DNeasy Mini Plant Kit	1
Proprietary	1
No dilution applied	1
Not specified	1

7. Real-time PCR quantification method(s)	No. of laboratories
a) EU-RL validated method(s)	66
b) In-house developed and optimised	3
c) International literature	1
d) ISO/CEN published method(s)	2
e) National reference method(s)	3
f) Other	4
Other of which	
Eurofins GeneScan:GMO Quant Event DAS-59122-7;GMO Quant Event RT73 Rapeseed Oilseed rape endogene (PEP): Reinhard Zeitler, Klaus Pietsch, Hans-Ulrich Waiblinger. Validation of real-time PCR methods for the quantification of transgenic contaminations in rape seed. European Food Research and Technology, European Food Research and Technology, Volume 214, Number 4: 346 – 351	1
Use TaqMan 2x MasterMix instead of single components in PCR setup	1
Hernàndez et al. (2004) J.Agric.Food.Chem. 52:4632-37 and Hern M. & al. (2001) J. Agric. Food Chem. 49: 3622-3627.	1

7.3. Real-time PCR quantification method used within the scope of your ISO/IEC 17025 accreditation?	No. of laboratories
Yes	51
No	24

7.4. Real-time PCR quantification method	No. of laboratories
Multiplex PCR	2
Singleplex PCR	73

8. Real-time PCR instrument	No. of laboratories
a) ABI 7000	1
b) ABI 7300	4
c) ABI 7500	31
d) ABI 7700	2
e) ABI 7900HT	17
f) ABI StepOne & StepOnePlus	1
g) BioRad icycler	2
h) Corbett Rotor-Gene 6000	3
i) Roche LightCycler 480	4
j) Roche LightCycler 2.0	1
k) Stratagene Mx3000/Mx3005	7
l) Stratagene Mx4000	0
m) Other	6

Other of which	
Biorad CFX96	4
QIAGEN Rotor Gene Q	1
Roche LightCycler 2.0	1

9. Real-time PCR Master Mix*	**No. of laboratories**
a) ABI TaqMan® Universal PCR master mix	45
b) ABI TaqMan® Universal PCR master mix, no AmpErase® UNG	4
c) ABI TaqMan® Fast Universal PCR master mix	1
d) ABI TaqMan® Gold with Buffer A	2
e) Eurogentec: qPCR MasterMix	2
f) Sigma JumpstartTM Taq ReadyMix TM	1
g) Qiagen: QuantiTect SYBR Green PCR kit	0
h) Qiagen: QuantiTect Probe PCR kit	6
i) Roche: FastStart TaqMan® Probe Master (Rox)	0
j) Roche: FastStart Universal Probe Master (Rox)	2
k) Diagenode: Universal Mastermix	2
l) Eurogentec MESA GREEN qPCR MasterMix Plus for SYBR® Assay	0
m) Eurogentec qPCR MasterMix for SYBR® Green	0
n) Eurogentec qPCR MasterMix	4
o) Fermentas: Maxima™ Probe/ROX qPCR Master Mix	2
p) Fermantas: MaximaTM SYBR® Green/ROX qPCR Master mix	0
q) Ampliqon: RealQ PCR 2 x Master Mix	0
r) Takara: SYBR®Premix Ex Taq™	0
s) Takara: Premix Ex Taq™	1
t) Propietary real-time PCR master mix	0
u) Other	11
Other of which	
AmpliTaq Gold with Buffer II (used for Acc 8 modul)	1
BIO-RAD IQ SIX FOR Singleplex ; BIO-RAD IQ Powermix for Multiplex	1
Roche fast start master hybridization probe	1
ABI TaqMan® PCR Core Reagent Kit	1
Amplification reaction mixture for DAS59122 according to the EU-RL method	1
Eurofins reaction mix	1
ТУ У 24.6-02568182-001:2011	1
MasterMixes for each event provided by GeneScan kits	1
QIAGEN Quantitect Multiplex PCR Kit	1
Roche: Lightcycler 480 Probes Master	1
5X Hot FIREPol Probe qPCR Mix Plus (No Rox) Solis Biodyne Cat.N°08-15-00020 (Bioconnect)	1

* Some laboratories used several types of real-time PCR master mix

9.2. Number of reagents (i.e. DNA, primers, probe, water, …) involved?	**No. of laboratories**

a) 5	21
b) 6	45
c) 7	2
d) 8	0
e) Other	5
Other of which	
9	1
10	1
13	1
14	1
15	1

Q 10.1 Sample intake (in ng) per real-time PCR	**No. of laboratories**
a) 0-50	14
b) 50-100	13
c) 100-200	32
d) > 200	3

Questions 10.2 to 10.5 only had to be answered, in case of different sample intakes.

Q 10.2 Sample intake (in ng) per real-time PCR	**No. of laboratories**
a) 0-50	2
b) 50-100	4
c) 100-200	2
d) > 200	3

Q 10.3 Sample intake (in ng) per real-time PCR	**No. of laboratories**
a) 0-50	3
b) 50-100	2
c) 100-200	4
d) > 200	0

Q 10.4 Sample intake (in ng) per real-time PCR	**No. of laboratories**
a) 0-50	2
b) 50-100	0
c) 100-200	0
d) > 200	0

Q 10.5 Sample intake (in ng) per real-time PCR	**No. of laboratories**

a) 0-50	1
b) 50-100	0
c) 100-200	0
d) > 200	0

11. Number of PCR replicates per test item (genomic DNA levels 1 and 2):	**No. of laboratories**
a) 1	1
b) 2	9
c) 3	31
d) 4	12
e) 5	1
f) 6	11
g) Other	12
Other of which	
8	3
9	1
10	3
12	5

12. Real time detection method(s) for quantification	**No. of laboratories**
a) MGB	0
b) Roche probe	0
c) Taqman probe	77
d) SYBR® Green	0
e) Other	0

13. Real-time PCR quantification method used?	**No. of laboratories**
a) DNA copy number standard curve using a dilution series	37
b) Mass/mass standard curve using a dilution series	36
c) Delta Ct method	8
d) Other	2

Q 14 Real-time PCR quantification method(s): slope(s) endogenous gene	**No. of laboratories per GM event**	
	GT73	**59122**
-4.1 ≤ slope < -3.6	12	6
-3.6 ≤ slope ≤ -3.1	48	57
-3.1 < slope < -2.6	2	3
Other	1	0

Q 15 Real-time PCR quantification method: GM trait gene	**No. of laboratories per GM event**	
	GT73	**59122**
-4.1 ≤ slope < -3.6	9	7

-3.6 ≤ slope ≤ -3.1	50	54
-3.1 < slope < -2.6	2	1
Other	1	0

Q 16 Real-time PCR quantification method(s): R^2 coefficient(s) endogenous gene	**No. of laboratories per GM event**	
	GT73	**59122**
$0.97 < R^2 < 0.98$	2	1
$0.98 \leq R^2 \leq 0.99$	7	3
$0.99 \leq R^2 \leq 1.00$	33	39
Other	2	1

Q 17 Real-time PCR quantification method: R^2 coefficient(s) GM trait gene	**No. of laboratories per GM event**	
	GT73	**59122**
$0.97 < R^2 < 0.98$	2	1
$0.98 \leq R^2 \leq 0.99$	7	11
$0.99 \leq R^2 \leq 1.00$	39	32
Other	1	1

Q 18. Real-time PCR quantification method(s): endogenous target DNA sequence(s)	**No. of laboratories per GM event**	
	GT73	**59122**
CruA	60	0
Hmg	0	55
Adh	0	15
Other	9	4

Q 19. Real-time PCR quantification method(s): GM trait target DNA sequence(s)	**No. of laboratories per GM event**	
	GT73	**59122**
P35S	0	3
P-FMV	0	0
T-E9	0	0
CP4-EPSPS	2	0
gox	0	0
gox247	0	0
P-ubiZM1	0	0
T-pinII	0	0
T-stpi	0	0
Cry35Ab1	0	0
P-Tap	0	0
Cry34Ab1	0	0
T-35S	0	0
T-nos	0	1

Pat	0	1
nptII	0	0
bar	0	0
CryIAb	0	0
GT73(R73) event specific	71	0
DAS59122 event specific	0	76
Other	1	1

20. Which reference material(s) was(were) used for calibration?	**No. of laboratories**
a) AOCS 0304-A	24
b) AOCS 0304-B	62
c) ERM-BF424	69
d) Dual-target plasmid(s)	1
e) Multiple target plasmid(s)	0
f) Other	87
Other of which	
AOCS 0306-G	1
ERM BF411a	1
Single target plasmids (pENGL-00-03/05-01; pENGL-00-26/04-01) provided by EU-RL	1
Single-target plasmid	1
Eurofins Genescan reference materials	1
RT-73 10% GM Sample prepared on laboratory	1
Internal powder GT73 100% homozygous	1
GT73 leaf DNA	1

21. Which reference material(s) was(were) used for quality control?	**No. of laboratories**
a) AOCS 0304-A	26
b) AOCS 0304-B	53
c) ERM-BF424	67
d) Dual-target plasmid(s)	0
e) Multiple target plasmid(s)	0
f) Other	89
Other of which	
AOCS 0306-G	1
AOCS 0306-B and ERM BF411a	1
Single target plasmids (pENGL-00-03/05-01; pENGL-00-26/04-01) provided by EU-RL	1
GT73 seeds from Institut für Hygiene und Umwelt der Hansestadt Hamburg	1
RT-73 10% GM Sample prepared on laboratory	1
Self prepared 0.1% standard for RT73 based on homozygous seeds	1
Self mixed 0.1% seed standard: 10 kernel GT73 (Monsanto,	

homozygote) in 10000 kernels	1
Plasmid DNA solution for GT73 (pGSE629)	1
Plasmid control for RT73 pENGL-00-26/04-01; for DAS pENGL-00-03/05-01	1
Old Proficiency samples	
Single-target plasmid	1
Positive samples	1
10 copies standard from Gene Scan (Eurofins Company)	1
Internal powder GT73 100% homozygous	1
Self mixed 0.1 % seed standard: 10 kernel GT73 (Monsanto, homozygote) in 10000 kernels	1
	1

Q 22. Practical LOD and LOQ (in %) of the GM content determination in mass/mass od DNA copy number ratio? Event GT73	**No. of laboratories**			
	LOD m/m	**LOD cp/cp**	**LOQ m/m**	**LOQ cp/cp**
0.001	1			
0.003		1		
0.005	1	1		
0.007	1			
<0.01	1		1	
0.01	12	5	1	1
0.02	4	2	1	1
0.03	3	1	1	
0.04	2	2	2	2
<0.05	1	1		
0.05	5	4	1	1
0.06				1
0.07			1	
0.08	2			3
0.09			2	
0.1	5	6	27	10
0.2			1	3
0.3			2	1
0.4	1		1	
4.9*	1			
6*		1		
10*		1		
14.85*		1		
68*				1

*Seem to be absolute values of DNA copies.

Q 22. Practical LOD and LOQ (in %) of the GM	**No. of laboratories**

content determination in mass/mass od DNA copy number ratio? Event 9122	LOD m/m	LOD cp/cp	LOQ m/m	LOQ cp/cp
0.005	1			
<0.1	3		1	
0.01	4	7	1	2
0.02	5	1		1
0.03	6	1		
0.04	5			
0.05	5	4	3	2
0.06	1	2	1	1
0.07	2			1
0.09		1	3	1
0.1	11	6	33	9
0.2	1		2	2
0.3			2	1
0.5			1	1
6*		1		
11*				1
14*		1		
20*		1		
57*				1

*Seem to be absolute values of DNA copies.

*Values 6 and 11 were obtained by L53, values 20, 57 by L37 and value 14 by L13

23. Did you report the uncertainty (*U*) as a relative value in % (i.e. does *U* correspond to a percentage of the reported GM level, e.g. *U* is equal to 25 % of the reported GM level)?	**No. of laboratories**
Yes	35
No	40

23.1. Does the uncertainty correspond to a relative repeatability standard deviation?	**No. of laboratories**
a) Yes	31
b) No	11
c) Not applicable	21

23.2. Does the uncertainty correspond to a relative within-laboratory reproducibility standard deviation?	**No. of laboratories**
a) Yes	13
b) No	24
c) Not applicable	22

23.4. Did you report an expanded uncertainty including a coverage factor?	**No. of laboratories**

a) Yes	36
b) No	8
c) Not applicable	21

23.5. If applicable, 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**
a) k = 1	1
b) k = 2	42
c) k = 3	0
d) k=2.78	1

24. Did you report the uncertainty as an absolute value?	**No. of laboratories**
Yes	36
No	39

24.1. Does the uncertainty correpond to a repeatability standard deviation?	**No. of laboratories**
a) Yes	23
b) No	13
c) Not applicable	24

24.2. Does the uncertainty correspond to a within-laboratory reproducibility standard deviation?	**No. of laboratories**
a) Yes	9
b) No	23
c) Not applicable	23

24.4. Did you report an expanded uncertainty including a coverage factor?	**No. of laboratories**
a) Yes	34
b) No	4
c) Not applicable	22

24.5. If applicable, 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**
a) k = 1	1
b) k = 2	36
c) k = 3	1

12. Acknowledgements

With respect to the raw material(s) used in this study we kindly acknowledge Pioneer Overseas Corporation for providing maize event 59122 and Monsanto Company Corporation for providing the oilseed rape event GT73. We sincerely thank Roberta Brustio, Stéphane Cordeil, Steven Price, Dario Pardi, Pierluigi Tenuta, of the MBG Unit and EU-RL GMFF for their invaluable contributions to this fifth comparative testing round. A special thanks to Marko Maras and Karolina Kolodziej who were very actively involved in the comparative testing activities. 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 laboratories listed below are kindly acknowledged for their participation in this exercise

Organisation	Department	Country	Status
A Bio Tech Lab D.O.O.		RS	4
AGES - Institute for Food Safety Vienna		AT	1, 2
Agricultural Institute of Slovenia		SI	2
Agri-Food and Veterinary Authority of Singapore	Laboratory Department	SG	4
Agroscope Liebefeld-Posieux Research station ALP	Analytics	CH	4
ANSES - Laboratoire de la santé des végétaux	Equipe OGM	FR	1, 2
BioGEVES		FR	1, 2
BIOMI Ltd		HU	3
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit		DE	1
Bureau of Plant Industry, Plant Quarantine Service, Post Entry Quarantine Station	Department of Agriculture	PH	4
Central Agricultural Office, Food and Feed Safety Directorate	Feed Investigation NRL	HU	1, 2
Central Agricultural Office, National Food Chain Safety Office, FFSD	GMO laboratory	HU	1, 2
Central Control and Testing Institute of Agriculture	Department of Molecular Biology	SK	1, 2
Centre de Recerca en Agrigenomica CRAG	Molecular Genetics (OMGs)	ES	3
Centre wallon de recherches agronomiques	Valorisation des productions	BE	1, 2
Centro Nacional de Alimentación (Agencia Española de seguridad alimentaria y nutricion)	Biotechnology Unit	ES	1, 2
Chemisches und Veterinäruntersuchungsamt Freiburg (CVUA Freiburg)	Gentechnik	DE	2
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)		DE	2
Crop Research Institute	Molecular Biology RLGMO	CZ	1, 2
Danish Veterinary and Food Administration	Lab. for Plant Diagnostics	DK	1, 2
Department of Chemistry Malaysia	GMO Unit	MY	4
DTU-Food, National Food Institute	Toxicology and Risk Assessment	DK	1, 2
Executive Environment Agency	Lab.	BG	3
Federal Institute for Risk Assessment (BfR)	Food Safety	DE	2
Federal Office of Public Health FOPH	Consumer Protection Directorat	CH	3

Finnish Customs Laboratory	ET2 / BIO	FI	1, 2
Food and Environment Research Agency (FERA)	Biotechnology molecular genetics	IE	1
Food and Environment Research Agency (FERA)	Biotechnology molecular genetics	UK	2
INRAN - Seed Testing Station	Laboratorio Analisi Sementi	IT	2
Institut für Hygiene 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 of Biochemistry and Biophysics PAS		PL	2
Institute of Food Safety, Animal Health and Environment „BIOR"	Virology	LV	1, 2
Institute of Molecular Genetics and Genetic Engineering	Plant Molecular Biology Lab	RS	4
Instituto Nacional de Recursos Biológicos (INRB)	Laboratório de Caracterização de Materiais de Multiplicação de Plantas	PT	2
Instytut Zootechniki PIB Krajowe Laboratorium Pasz Pracowania w Szczecinie		PL	1, 2
Istituto Superiore di Sanità	DSPVSA	IT	2
Istituto Zooprofilattico Sperimentale della Sardegna	Igiene degli alimenti	IT	5
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Biotecnologie	IT	1, 2
Istituto Zooprofilattico Sperimentale delle Venezie	Microbiologia degli alimenti	IT	5
Kyung Hee University	Food Science & Biotechnology	KR	4
Laboratoire national de santé	Food control	LU	1, 2
Laboratorio Arbitral Agroalimentario - MAGRAMA	OGM	ES	1, 2
Landesamt für Umweltschutz	FG13	DE	2
Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	DE	2
Landesbetrieb Hessisches Landeslabor		DE	2
Landeslabor Berlin-Brandenburg	Fb 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 Lebensmitteluntersuchung	DE	2
LAVES-State Food and Veterinary Laboratory Braunschweig/Hannover	FB 120 Molecular Biology	DE	2
LGC Limited	Molecular and Cell Biology	UK	1, 2
LTZ Augustenberg		DE	2
Ministry of Finance, General Secretariat for Tax and Customs Issues, General Chemical State Laboratory (GCSL)	Food Division Athens	GR	1, 2
National Bureau of Plant Genetic Resources	NRC on DNA Fingerprinting	IN	4
National Center of Public Health and Analyses	Bulgarian National Laboratory for Genetically Modified Food	BG	1, 2
National Food Agency	Science Department	SE	1, 2
National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO Section	LT	1, 2
National Institute of Biology	Department of Biotechnology	SI	1, 2
National Public Health Laboratory, Ministry of Health	Food Department	MY	4
National Veterinary Institute	Food Bacteriology and GMO	NO	3
National Veterinary Research Institute	Hygiene of Feed	PL	1, 2
Netherlands Food and Consumer Product Safety Authority		NL	2

Plant Breeding and Acclimatization Institute – National Research Institute	GMO Controlling Laboratory	PL	2
RIKILT	NFA	NL	1, 2
Science and Advice for Scottish Agriculture (SASA)		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
Servizio Fitosanitario e chimico	ERSA - Regione autonoma Friuli Venezia Giulia	IT	5
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Geschäftsbereich 6, FB 63	DE	2
State Office for Agriculture, Food Safety and Fishery Mecklenburg-Western Pomerania		DE	2
State Veterinary and Food Institute Dolny Kubin	Department of molecluar biology analysis	SK	1, 2
Tallinn University of Technology	Gene Technology	EE	2
Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (TLLV)	Lab for detection of GMO/foods	DE	2
Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	DE	3
UkrMetrTestStandard	Molecular-diagnostic lab	UA	4
Umweltbundesamt GmbH		AT	1, 2
USDA, Grain Inspection Packers Stockyards Administration (GIPSA), Tech. Services Division	Biotechnology	US	4

[1] Laboratory appointed under Regulation (EC) No 882/2004, [2] Laboratory appointed under Regulation (EC) No 1981/2006, [3] ENGL member only, [4] Laboratory from third country, [5] Official control laboratory only

13. Annex 1: Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Molecular Biology and Genomics

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 24 April 2012
JRC.DG.I.3/MBG/JK/DC/dp/ARES(2012)507387

NOTE FOR THE ATTENTION OF

I. **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 882/2004**
II. **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 1981/2006**
III. **All members of the European Network of GMO Laboratories**
IV. **Official control laboratories**
V. **Interested parties from third countries**

Subject: Invitation to participate in the comparative test ILC-EURL-GMFF-CT-01/12

Pursuing Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004, the European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF) has the obligation to organise comparative testing rounds and to ensure an appropriate follow-up of the results obtained.

Hereby, I would like to invite you to participate in the fifth round of comparative testing ILC-EURL-GMFF-CT-01/12. This round of comparative testing will include two test materials of a genomic DNA solution. The participant will need to quantify the GM content of maize event 59122 and of oilseed rape line RT73 in each test material.

Your participation is free of charge. Participants in the comparative testing rounds need to dispose over equipment for qualitative and quantitative Polymerase Chain Reaction (PCR).

I would like to remind you that participation in comparative testing is mandatory for all National Reference Laboratories nominated under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006. The laboratory codes assigned to each participant in this comparative testing round are confidential. However, the EU-RL 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.

Comparative testing is organised by the EU-RL GMFF in collaboration with the Institute for Reference Materials and Measurements (IRMM, Geel, BE).

Joint Research Centre · I-21027 Ispra (VA), Italy · TP 331
Telephone: direct line (+39)0332/786735, · Telefax: (+39)0332/785483
E-mail: Joachim.kreysa@ec.europa.eu
http://ihcp.jrc.ec.europa.eu

ISO 9001:2008 certified by
CERMET SISTEMA DI GESTIONE CERTIFICATO
SINCERT

Registration for the fifth round of comparative testing and submission of results will be handled by IRMM. Please register electronically for the fifth comparative testing round using the following link:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=880

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: +32 14 571 865
Mail: JRC-IRMM-IMEP@ec.europa.eu
Cc to: mbg-comparative-testing@jrc.ec.europa.eu

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

The deadline for registration is **10 May 2012**. Samples should be shipped during the week of **28 May to 1 June 2012**. The provisional deadline for submission of results is **13 July 2012**.

Please contact JRC-IRMM-IMEP@ec.europa.eu and JRC-IRMM-MILC@ec.europa.eu **ONLY** for difficulties with your on-line registration.
For **all other issues** (communications, questions related to the content of the comparative testing round) please contact:

Diana Charels
European Commission – Joint Research Centre
Molecular Biology and Genomics Unit – TP201
Via E. Fermi 2749
I-21027 Ispra (VA)
Phone: +39 0332 78 6518
Fax: +39 0332 78 9333
E-mail: mbg-comparative-testing@jrc.ec.europa.eu

The EU-RL GMFF is looking forward to your participation.

Yours sincerely,

Joachim Kreysa
Head of Molecular Biology and Genomics Unit

Copy: M. Mazzara, D. Charels, E. Nardini, C. Savini, K. Kolodziej, M. Maras, F. Ulberth (JRC).

14. Annex 2: Accompanying letter

Ref. Ares(2012)622234 - 24/05/2012

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 24 May 2012
JRC.DG.I.3-MBG/JK/EN/dp/ARES(2012)

NOTE FOR THE ATTENTION OF

«Address»

Subject: Participation in ILC-EURL-GMFF-CT-01/12, a comparative testing round to determine the GM content in two test materials of a genomic DNA solution.

Dear «Name» «Surname»,
Thank you for participating in the ILC-EURL-GMFF-CT-01/12 comparative testing round containing two test materials of a genomic DNA solution.

You will receive the test items shipped in dry ice via courier. The shipment will be carried out in the week of **4 to 8 June 2012**. 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 screw-cap tubes (level 1 and level 2) each containing a genomic DNA solution at 80ng/uL
2. An "Acknowledgement of Reception" form
3. This accompanying letter

Please check whether screw-cap tubes containing the test material remained undamaged during transport and return the "Acknowledgement of Reception" form by fax (+39 0332 789333). You should store the samples at -20ºC.

This round of comparative testing will include two test materials of a genomic DNA solution. The participant will need to quantify the GM content of maize event 59122 and of oilseed rape line RT73 in each test material.

There is no need to quantify the genomic DNA solution received. It is suggested to use 2.5uL of this genomic DNA solution for each PCR reaction. 2.5uL contain 36697 maize genome copies and 86956 oilseed rape genome copies.

The procedures used for detection/quantification of the detected GM events should resemble as closely as possible the ones that you use in routine sample analyses.

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

ISO 9001:2008 certified by

Joint Research Centre · 21027 Ispra VA, Italy · TP 201
Secretariat · Phone: +39 0332 789379, Fax: +39 0332 785483
E-mail: JRC-BGMO@ec.europa.eu
WWW: http://gmo-crl.jrc.ec.europa.eu/ · http://bgmo.jrc.ec.europa.eu · http://ihcp.jrc.ec.europa.eu

CERMET SISTEMA DI GESTIONE CERTIFICATO SINCERT

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

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

You can find the reporting website at https://web.jrc.ec.europa.eu/ilcReportingWeb . You need a personal password to access this webpage which is «PARTKEY». The system will guide you through the reporting procedure.

After entering all results, please complete the questionnaire. In the questionnaire, items bearing a question mark icon on the right-hand sidecontain 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 was sent 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 that are reported on-line on the reporting website https://web.jrc.ec.europa.eu/ilcReportingWeb **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 **20 June 2012**. It will not be possible to submit your results after the deadline.

Please contact JRC-IRMM-IMEP@ec.europa.eu and JRC-IRMM-MILC@ec.europa.eu **ONLY** for reporting difficulties, failures or anomalies with the online system for reporting.
For **all other issues** (communications, questions related to the content of the comparative testing round) please contact:

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

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

Yours sincerely,

Joachim Kreysa
Head of Molecular Biology and Genomics Unit

15. Annex 3: Confirmation of shipment

Reference: JRC.DG.I.3-MBG/JK/dp/ARES(2012)661856

Dear Participant,
All test parcels related to the fifth round of comparative testing have left our premises on June 06 by TNT **courier**. For your convenience, please find herewith the corresponding airway bill number you could refer to in order to track the relevant materials on the Web:
251109271

The parcel with test items that you will or have already received should contain:

1. **Two test materials of genomic DNA**, both containing oilseed rape GT73 and maize DAS 59122.
2. An **acknowledgement of reception** form, that should be returned to the EU-RL GMFF by fax (+39 0332 789333). In case you did not yet receive the test items please contact Dario PARDI (Dario.PARDI@ec.europa.eu; phone +39 0332 78 51 65).
3. An accompanying letter entitled '**Participation in ILC-EURL-GMFF-CT-01/12'.**

The accompanying letter contains your **personal password** for on-line submission of your results to the reporting website https://web.jrc.ec.europa.eu/ilcReportingWeb/.

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. 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://web.jrc.ec.europa.eu/ilcReportingWeb will be accepted.

The deadline for submission of your results is **20 July 2012.**

Please contact JRC-IRMM-IMEP@ec.europa.eu and JRC-IRMM-MILC@ec.europa.eu **ONLY** for reporting difficulties, failures or anomalies of the online system for reporting (i.e. https://web.jrc.ec.europa.eu/ilcReportingWeb). For **all other issues** (communications, questions related to the content of the comparative testing round) please contact:

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

Please send me an e-mail (mbg-comparative-testing@jrc.ec.europa.eu) in case you have not received the above-mentioned documents.

Thank you

Dario PARDI
Secretariat

European Commission
DG Joint Research Centre
Institute for Health and Consumer Protection
Unit I.03 Molecular Biology and Genomics
TP 201 Via E**.** Fermi 2749
I-21027- Ispra (VA) Italy
+39 0332 78.5988

16. Annex 4: Acknowledgement of receipt

FAX - Record for Quality System

JRC.I.4 -MV

Date: **R71GP6/EURL** 19/07/2011 **Acknowledgement of reception**
Page 1/1

Revision. 4

From :

Lab Code:

To : Molecular Biology and Genomics Unit fax: +39 0 332 78 6159
Method Validation / EURL-GMFF
European Commission - Joint Research Centre - IHCP
21027 ISPRA (VA) Italy File nb EURL-CT-01/12

We have received the following samples	**In good condition Yes**	**No**
Two test tubes (Level 1 and Level 2) of a genomic DNA solution delivered in dry-ice		

No information regarding the sample(s) received and results of related testing may be disclosed to any third party.

Comments:

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

By signing this document the participant agrees with the clause of non disclosure of information on samples and results

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.

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European Commission
EUR 26188 EN – Joint Research Centre – Institute for Institute for Health and Consumer Protection

Title: Comparative Testing Report on the Quantification of Maize Line DAS-59122-7 and Oilseed rape Line GT73 (RT73): ILC-EURL-GMFF-CT-01/12

Author(s): Diana Charels, Luminita Dascalu, Niccolo Bassani, Adam Niedzwiecki, Fernando Cordeiro Raposo, Inge Verbist, Marco Mazzara

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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 fifth comparative testing round ILC-EURL-GMFF-CT-01/12. Participants had to determine the content of oilseed rape event GT73 and maize event 59122 in two test items denoted genomic DNA levels 1 and 2, containing different GM percentages of both GM events.

This comparative testing round was organised in collaboration with the Food Safety and Quality Unit of the Institute for Reference Materials and Measurements (Geel, BE). The test items were produced in-house. The Food Safety and Quality Unit managed the on-line registration and submission of results.

A total of 160 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/12. Eighty laboratories from 36 countries returned results, of which 59 were National Reference Laboratories, six were only members of the European Network of GMO Laboratories, three were only Official control laboratories and 12 were laboratories from third countries. Five laboratories including one National Reference Laboratory and four laboratories from third countries did not submit results.

In this fifth comparative testing round 92 % to 98 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in mass/mass % depending on the GM content and the GM event. However, a lower percentage (38 – 93 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in copy/copy %.

LB-NA-26188-EN-N

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Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security, including nuclear; all supported through a cross-cutting and multi-disciplinary approach.

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