

JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS

# Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604

Comparative testing round: ILC-EURL-GMFF-CT-02/11 version b

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The corrections made in the new document are:

Page 25 Table 4 : Uncertainty relative for L015 (a) (c) 0.44 Changed in (a) (c) 0.06

Page 26 Table 5: Uncertainties were sorted according to laboratory number and the columns regarding absolute and relative uncertainty updated.

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# Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604

Comparative testing round: ILC-EURL-GMFF-CT-02/11

Date of issue version b: 10 June 2013

### Joint Research Centre

Institute for Health and Consumer Protection Molecular Biology and Genomics Unit

Report number: EURL-CT-02/11 CTRb Status: Recalled final report

Motivation: The uncertainty estimations depicted in Tables 4 and 5 have been corrected following an appeal.

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.

ISO 17043 Accreditation Proficiency Test Provider by:



Correction from the previous version:

#### version b - 10/06/2013

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**Page 26 Table 5** : Uncertainties were sorted according to laboratory number and the columns regarding absolute and relative uncertainty updated.

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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<sup>(1)</sup>, organised a comparative testing round for National Reference Laboratories (NRLs) nominated under Regulation (EC) No 882/2004<sup>(2)</sup> and Regulation (EC) No 1981/2006<sup>(3)</sup>, 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 was in accordance with the ISO 17043 Standard<sup>(4)</sup>. The EU-RL GMFF is accredited according to the ISO 17043 Standard 'General requirements for proficiency testing'<sup>(4)</sup>.

The test items used in the comparative testing round ILC-EURL-GMFF-CT-02/11 were produced in-house. Pioneer Overseas Corporation provided a coarsely ground powder of TC1507 (unique identifier DAS-Ø15Ø7-1) seeds. Syngenta Biotechnology, Inc. provided devitalised seeds of maize events MIR604 (unique identifier SYN-IR6Ø4-5) and GA21 (unique identifier MON-ØØØ21-9). Participants were required to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. Any events detected were then to be quantified. In September 2011, a total of 159 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11, and subsequently 102 laboratories registered for this comparative testing round. Test items were shipped to participants at the end of October 2011 in plastic containers containing approximately 5 g of flour. Ninety-three laboratories from 40 countries returned results, which fell into the following groups:

- 1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1),
- 2. 29 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
- 3. 30 were NRLs nominated under both Regulations (group 3),
- 4. 7 were only ENGL members (group 4),
- 5. 8 were only official control laboratories (group 5),
- 6. 16 were laboratories from third countries (group 6).

Eight laboratories, of which seven were NRLs (groups 1 to 3) and one was an official control laboratory (group 5), submitted results in both measurement units. Two NRLs and one ENGL member (group 4) registered twice but submitted both sets of results in the same measurement unit. Nine laboratories including one NRL (group 3), one ENGL member and

seven laboratories from a third country (group 6) did not submit results. 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.

Participants could report the results in either mass/mass % (m/m %) or copy/copy % (cp/cp %). The EU-RL GMFF calculated the robust means ( $\mu_R$ ) of the maize powder 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 <sup>(5, 6, 7)</sup>. In addition, values ( $\mu$ ) were assigned by the EU-RL GMFF on the basis of the data from the homogeneity study<sup>(8)</sup> (m/m % data) and digital Polymerase Chain Reaction (PCR)<sup>(9)</sup> (cp/cp % data). The homogeneity, stability and digital PCR studies were conducted at the EU-RL GMFF. These data were included in the uncertainty budget.

The target standard deviation for comparative testing  $\sigma$  was fixed at 0.20 (log<sub>10</sub> value) for the maize events TC1507 and MIR604 and 0.25 (log<sub>10</sub> value) for event GA21 by the Advisory Board for Comparative testing. These target standard deviations were used to derive z-scores for the participants' results. An overview of the assigned values, robust means and number of z-scores in the range of -2 to +2 is given in Figure 1.



**Figure 1**: Overview of z-scores calculated on the basis of assigned values and robust means for maize events GA21 (a), TC1507 (b) and MIR604 (c). m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, L1 = level 1, L2 = level 2.



**Figure 1 (continued)**: Overview of z-scores calculated on the basis of assigned values and robust means for maize events GA21 (a), TC1507 (b) and MIR604 (c). m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, L1 = level 1, L2 = level 2.

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in m/m % for both maize powder levels 1 and 2 regardless of the GM event. However, a lower percentage (43 – 86 %) 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 only about 53 % of participants provided information on measurement uncertainty in a complete and consistent manner.

# Content

1.	Introduction	9
2.	Description of the comparative test items	
2.1	Preparation	
2.2	Purity testing	
2.3	Homogeneity and stability assessment	11
3.	Participants' results	12
4.	Reporting of results	18
5.	Assigned value and measurement uncertainty	
5.1	Reference values determined by the test item producer	
5.2	Consensus values from participants	
6.	Statistical data and summaries	23
7.	Interpretation of z-scores	49
8.	Evaluation of results	49
9.	Performance of laboratories	53
9.1	NRLs	53
9.2	Non-NRLs	55
9.3	Results of the repetition of the experimental work	
9	9.3.1 NRLs	
10	Conclusions	00 60
11	References	62
17	Auastiannaira data	6 <i>1</i>
12.		
13.	Acknowledgements	79
14.	Annex 1: Invitation letter	83
15.	Annex 2: Accompanying letter	85
16.	Annex 3: Confirmation of shipment	
17.	Annex 4: Acknowledgement of receipt	
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# 1. Introduction

The Joint Research Centre (JRC) as European Union Reference Laboratory for Genetically Modified Food and Feed (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 National Reference Laboratories (NRLs) and shall ensure an appropriate follow-up of such testing. The aim of this activity is 'to contribute to a high quality and uniformity of analytical results<sup>(2)</sup>. Moreover, Article 12 of Regulation (EC) No 882/2004 states that the nominated NRLs should be accredited in accordance with ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. One of the requirements of ISO/IEC 17025 accredited laboratories is to prove their competence by taking part in a proficiency testing scheme.

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

In 2011 the EU-RL GMFF organised the fourth comparative testing round in collaboration with the Food Safety and Quality (FSQ) Unit of the Institute for Reference Materials and Measurements (IRMM). The comparative testing round was announced at the European Network of GMO Laboratories (ENGL) plenary meeting on the 24<sup>th</sup> and 25<sup>th</sup> of May 2011. In September 2011, a total of 159 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11, and subsequently 102 laboratories registered for this comparative testing round. Test items were shipped to participants at the end of October 2011 in plastic containers containing approximately 5 g of flour. Ninety-three laboratories from 40 countries returned results, which fell into the following groups:

- 1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1),
- 2. 29 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
- 3. 30 were NRLs nominated under both Regulations (group 3),
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Eight laboratories, of which seven were NRLs (groups 1 to 3) and one was an official control laboratory (group 5), submitted results in both measurement units. Two NRLs and one ENGL member (group 4) registered twice but submitted both sets of results in the same measurement unit. Nine laboratories including one NRL (group 3), one ENGL member and

seven laboratories from a third country (group 6) did not submit results. The FSQ Unit of IRMM managed the on-line registration and submission of results.

# 2. Description of the comparative test items

#### 2.1 Preparation

Test items were prepared in the EU-RL GMFF in accordance with ISO Guide 34<sup>(10)</sup> regarding the 'General requirements for the competence of reference material producers'.

Maize powder levels 1 and 2 were prepared to nominal values of 0.3 m/m % and 2.2 m/m % GM of GA21, 0.7 m/m % and 2.0 m/m % GM of TC1507, 3.5 m/m % and 1.0 m/m % GM of MIR604 flours, respectively.

The preparation of test items was carried out between the end of July and the end of September 2011. Raw materials (seeds) were assessed for basic seed traits (i.e. water content) and for the presence of other GM events authorised within the European Union. The zygosity of the events GA21, TC1507 and MIR604 was assessed in the GM line. Powder of TC1507 was prepared by a one-step grinding process using an Ultra Centrifugal Mill ZM200 (Retsch GmbH, DE). Powders of non-modified and events GA21 and MIR604 maize were preground using a GM200 knife mill (Retsch GmbH, DE) prior to the one-step grinding process using the Ultra Centrifugal Mill ZM200. All powders were tested for DNA extractability using the Macherey-Nagel (Düren, DE) plant DNA extraction kit, and a validated CTAB DNA extraction method, to demonstrate equal extractability of all powders. Test items were then prepared in a one-step dilution by dry-mixing non-modified maize powder and GA21, TC1507 and MIR604 maize powders in specified mass proportions corrected for the water content.

Approximately 5 g of the test items were aliquoted in 30-mL plastic tubs using an automatic sampling device, and labelled as maize powder levels 1 or 2. Test items were stored at +4  $^{\circ}$ C in the dark.

### 2.2 Purity testing

Purity tests conducted at the EU-RL GMFF detected the adventitious presence of maize events NK603, MON 810 and 59122 below the Limit of Quantification (LOQ). Table 1 lists the GM events present in maize powder levels 1 and 2. Different quantities of the maize events GA21, TC1507 and MIR604 were added to both test items.

GM event	Maize powder level 1	Maize powder level 2
GA21	+	+
TC1507	+	+
MIR604	+	+
NK603	Adv	Adv
59122	Adv	Adv
MON 810	Adv	Adv
MON 863	-	-
3272	-	-
Bt11	-	-
Bt176	-	-

**Table 1**: Overview of GM events present in maize powder levels 1 and 2. + indicates that the GM event was added to the test items, - indicates that the GM event was not added to the test items, Adv = adventitious presence

#### 2.3 Homogeneity and stability assessment

The assessment of the homogeneity was performed after the test items had been packed in their final form and before distribution to participants<sup>(11)</sup>.

Samples are considered to be adequately homogeneous if:

$$s_s \le 0.3 \sigma$$
 (1)

where:  $s_s$  is the between-test item standard deviation as determined by a single factor ANOVA<sup>(12)</sup> and  $\overset{\circ}{\sigma}$  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

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

where: *MS*<sub>between</sub> is the mean sum of squares between test items *MS*<sub>within</sub> is the mean sum of squares within test items *n* is the number of replicates

 $\overline{y}$  is the mean of the homogeneity data

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

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

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

For each GM level ten test items (N = 10) were randomly selected and analysed in five-fold replicates (n = 5). The criterion described in formula (1) was fulfilled thus indicating that both maize powder test items were homogeneous.

The data from the homogeneity study conducted at the EU-RL GMFF were used for the estimation of the uncertainty contributions related to the homogeneity of the maize powder 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 °C, +18 °C and +60 °C<sup>(13)</sup>. The results of the study did not reveal any influence of time and temperature on the stability of test items, and therefore it was concluded that the test items could be shipped to participants at ambient temperature.

An isochronous long term stability study involving two maize powder level 1 test items (N = 2, n = 3) was conducted for time periods of three, six and ten months at a temperature of +4 °C<sup>(13)</sup>. No significant trend (95 % confidence level) was detected for any of the GM events tested thus indicating that test items can be stored at +4 °C.

## 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 screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. Any events detected were then to be quantified. 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<sup>(14)</sup>, 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.

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

A total of 93 laboratories from 40 countries reported results (Figures 2 and 3).

EURL-CT-02/11final CTRb



Figure 2: Distribution of participants from different countries

A majority of laboratories reported the GM content in m/m % (Figure 4). Two laboratories registered twice and submitted both sets of results in m/m %. A minority of laboratories expressed their results in cp/cp % (Figure 4) of which four laboratories (L028, L066, L074 and L095) used dual-target plasmids. All other laboratories used a genomic DNA calibrant: Certified Reference Materials (CRMs) from IRMM. One laboratory registered twice and submitted both sets of results in cp/cp %. A few laboratories reported the results in both measurement units (Figure 4).



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



**Figure 4**: Overview of participants' results grouped by GM event GA21 (a), TC1507 (b), MIR604 (c) and measurement unit. m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, Both = results submitted in both measurement units, L1 = level 1, L2 = level 2.



**Figure 4 (continued)**: Overview of participants' results grouped by GM event GA21 (a), TC1507 (b), MIR604 (c) and measurement unit m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, Both = results submitted in both measurement units, L1 = level 1, L2 = level 2.

The EU-RL GMFF calculated the robust means ( $\mu_R$ ) of the maize powder levels 1 and 2 test items in m/m % and cp/cp %. All data were log-transformed and then robust statistics were applied to obtain a robust mean<sup>(5, 6, 7)</sup>. In addition, values ( $\mu$ ) were assigned by the EU-RL GMFF on the basis of the data from the homogeneity study<sup>(8)</sup> (m/m % data) and digital Polymerase Chain Reaction (PCR)<sup>(9)</sup> (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 4 to 15. An overview of the analytical methods used by each participant is summarised in section 12 'Questionnaire data'.

# 4. Reporting of results

Purity tests conducted at the EU-RL GMFF detected the adventitious presence of maize events NK603, MON 810 and 59122 below the Limit of Quantification (LOQ). Table 1 lists the GM events present in maize powder levels 1 and 2. Different quantities of the maize events GA21, TC1507 and MIR604 were added to both test items.

Nine percent of participants (L064, L075, L086, L091, L092, L093, L094 and L098) of which 2 % were NRLs (L064 and L086) only performed screening analyses. At least 91 % of partipants detected maize events GA21 and TC1507, whereas about 80 % detected event MIR604 (Figure 5). About 18 % of participants of which 2 % were NRLs (L010 and L064) did not screen for event MIR604. One NRL (L064) did not screen for event GA21 and two NRLs (L010 and L064) did not screen for TC1507. L064 only screened for events Bt11, Bt176, MON 810 and NK603. Three out of 62 NRLs (L010, L029 and L084) did not quantify all three GM events. At most 3 % of participants did not detect the GM events GA21, TC1507 and MIR604 (i.e. false negative result, Figure 5). One NRL (L045) and two non-NRLs (L092, L093) reported false negative results. It is suspected that the false negative result reported by L045, regarding the screening of the GA21 event in the maize powder level 2 test item, is a reporting mistake because L045 quantified the GA21 content of the level 2 test item (Table 5). One NRL (L056) and four non-NRLs (L013, L022, L091, L092) reported false positive results. With respect to the adventitious presence of GM events about 77 % of participants detected event NK603, whereas 1 % and at most 5 % detected events 59122 and MON 810, respectively. The majority (about 65 % and 56 % for the results expressed in m/m % and cp/cp %, respectively) of participants reported results for the event NK603 in a semiquantitative way (i.e. < value x). Of those participants that quantified the GM content of event NK603 most participants (i.e. about 82 % and 75 % for the results expressed in m/m % and cp/cp %, respectively) reported a value below 0.1 %. Of those participants that detected events 59122 and MON 810, only one (L068) and two (L068 and L073) participants respectively, reported quantitative results for these GM events. Events 3272 and Bt176 were detected by none of the participants whereas at most 5 % and 1 % detected events MON 863 and Bt11 respectively. It can thus be concluded that a majority of participants performed a correct screening with a minority of participants reporting either false positives or negatives (Figure 5).

EURL-CT-02/11final CTRb



Figure 5a: Overview of screening data (in %) for maize powder level 1. D = detected, FN = : false negative, NS = not screened, FP = false positive, ND = not detected.

EURL-CT-02/11final CTRb



**Figure 5b**: Overview of screening data (in %) for maize powder level 2. D = detected, FN = : false negative, NS = not screened, FP = false positive, ND = not detected.

# 5. Assigned value and measurement uncertainty

#### 5.1 Reference values determined by the test item producer

Following evaluation of the data in the preliminary report 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. The assigned value in m/m % ( $\mu$ ) was derived from the homogeneity data (N = 10, n = 5)<sup>(8)</sup>. The assigned value in cp/cp % ( $\mu$ ) was determined by digital PCR (N = 5, n = 5)<sup>(9)</sup>.

The information relating to the EURL-GMFF-CT-02/11 maize powder levels 1 and 2 test items is outlined in the Table below.

**Table 2:** Assigned value ( $\mu$ ) and expanded uncertainty (U) of maize powder levels 1 and 2. <sup>1</sup>Relative standard uncertainty relating to the characterisation, <sup>2</sup>Relative standard uncertainty resulting from the homogeneity assessment, <sup>3</sup>Relative standard uncertainty resulting from the long-term stability assessment

"[m/m %]		Relative standa	rd uncertainty cor	ntributions [%]	Expande (U	ed uncertainty = 2 * u <sub>c</sub> )
$\mu$ [1171	1 20]	$(u_{char, rel})^1$	$(u_{bb, rel})^2$	$(u_{lts, rel})^3$	U <sub>rel [%]</sub>	U <sub>abs</sub> [m/m %]
GA21						
Level 1	0.26	4.09	3.62	9.09	21	0.06
Level 2	2.08	3.55	3.95	9.09	21	0.44
TC150	)7					
Level 1	0.30	3.46	2.25	6.55	15	0.05
Level 2	0.89	3.03	1.47	6.55	15	0.13
MIR60	)4					
Level 1	3.38	2.68	1.08	6.35	14	0.47
Level 2	0.89	4.95	5.94	6.35	20	0.18
μ [cp/cp	»%]					
GA21						
Level 1	0.14	10.60	3.62	9.09	29	0.04
Level 2	0.86	4.63	3.95	9.09	22	0.19
TC150	)7					
Level 1	0.19	4.84	2.25	6.55	17	0.03
Level 2	0.43	3.68	1.47	6.55	15	0.07
MIR604						
Level 1	1.34	4.88	1.08	6.35	16	0.22
Level 2	0.34	4.49	5.94	6.35	20	0.07

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 ( $u_{ts}$ )<sup>(15)</sup>. 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<sup>(16)</sup>.

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

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

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

where:  $\sigma$  = relative standard deviation of the mean

N = number of data points

The assigned values of maize powder levels 1 and 2 expressed in m/m % are traceable to the International System of Units (SI). The traceability chain is based on the use of calibrated balances and a thorough control of the weighing procedure.

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

#### 5.2 Consensus values from participants

The consensus value ( $\mu_R$ ) from participants in the comparative testing round was calculated using robust statistics<sup>(17)</sup>. 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<sup>(6)</sup>. Robust means ( $\mu_R$ ) 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<sup>(15)</sup> (Formula 6).

The robust means ( $\mu_R$ ) determined by the EU-RL GMFF are depicted in Table 3.

		Expanded	<u>uncertainty</u>
$\mu_{\rm R}$	[m/m %]	( <i>U</i> =	2 * u <sub>c</sub> )
-		U <sub>rel [%]</sub>	<i>U<sub>abs</sub></i> [m/m %]
	GA21		
Level 1	0.26 (N = 66)	22	0.06
Level 2	1.92(N = 67)	22	0.45
•	TC1507		
Level 1	0.38 ( <i>N</i> = 64)	16	0.05
Level 2	1.07 (N = 63)	15	0.13
Ν	MIR604		
Level 1	3.15 ( <i>N</i> = 58)	15	0.49
Level 2	0.91 (N = 58)	19	0.17
$\mu_{R}$ [	cp/cp %]		
	GA21		
Level 1	0.31 ( <i>N</i> = 24)	42	0.13
Level 2	1.76 (N = 26)	40	0.70
-	TC1507		
Level 1	0.26 (N = 29)	26	0.07
Level 2	0.71 (N = 29)	23	0.16
Ν	MIR604		
Level 1	2.57 (N = 23)	31	0.79
Level 2	0.85 ( <i>N</i> = 23)	37	0.31

**Table 3**: Overview of robust means ( $\mu_R$ ) and expanded uncertainties for maize powder levels 1 and 2

The standard uncertainty ( $u_{char}$ ) of the characterisation tends to increase when the robust mean is calculated on the basis of a lower number of data points (Formula 7).

## 6. Statistical data and summaries

The aim of a performance statistic is to provide participants with a meaningful result that can be easily interpreted. The procedure followed for the evaluation of participants' performance was agreed by the Members of the Advisory Board and relies on the calculation of z-scores on the basis of the assigned values<sup>(8, 9)</sup> ( $\mu$ ) and the robust means<sup>(17)</sup> ( $\mu_R$ ) of the participants' results.

Laboratories are compared on the basis of z-scores calculated from log-transformed data<sup>(6)</sup>. The z-scores are based on the assigned values ( $\mu$ ) and the robust means ( $\mu_R$ ) of the submitted results (Tables 4 to 15). 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 = 4).

EURL-CT-02/11final CTRb

The value of  $\sigma$ , 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 events TC1507 and MIR604 and 0.25 for event GA21<sup>(18)</sup>. The z-score (*z*) for participant / reporting measurement result *x*<sub>i</sub> is thus calculated as

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

where:  $\mu$  = assigned value expressed in m/m % or cp/cp %

$$z_i = \left(\log_{10} x_i - \log_{10} \mu_R\right) / \overset{\wedge}{\sigma} \tag{9}$$

where:  $\mu_R$  = robust mean expressed in m/m % or cp/cp %

**Table 4**: z-scores for event GA21 maize powder level 1 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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 underestimated (f) seems to be a typing mistake. Results are as submitted by participants.

	Maize event GA21						
			Robust me	an = 0.26 m	/m %		
Laboratory			Assigned v	alue = 0.26 i	n/m %		
number	Value	Uncert	ainty	LOD m/m	LOQ m/m	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L002	0.26		(a) 0.02	-	-	-0.01	0.00
L003	0.21	() 0.07	0.12	0.04	0.10	-0.38	-0.37
L004	0.25	(c) 0.07	0.40	-	-	-0.07	-0.07
L005	0.67		(b) 0.13	0.05	0.20	1.64	1.64
1 007	0.20		(b) 0.18 (b) 0.25	- 0.10	- 0.10	1.26	1 27
L008	0.54		(b) 0.35 (a) 0.36	< 0.10	0.10	1.20	1.27
L009	0.94	(e) 2 77	(a) 0.00	-	-	2.23	2.23
L011	0.10	(0) 2.11	(a) 0.05	0.01	0.10	-1.67	-1.66
L012	0.58		(a) 0.00	0.10	0.10	1.39	1.39
L013	0.25		0.11	0.02	0.08	-0.07	-0.07
L015	0.20	(a) (c) 0.06		0.01	0.05	-0.46	-0.46
L016	0.27		(a) 0.09	0.10	0.10	0.06	0.07
L017	0.28	70.00		0.08	0.08	0.12	0.13
L018	0.17		(a) 0.08	-	-	-0.74	-0.74
L019	0.17		0.08	-	-	-0.74	-0.74
L020	0.16	= / 00	0.04	0.01	0.11	-0.85	-0.84
L021	0.18	74.00		-	-	-0.64	-0.64
L023	0.21		0.06	0.03	0.10	-0.38	-0.37
L024	< 0.20	(a) 0.00	-	-	-	0.54	0.52
L025	0.55	(0) 0.00	(a) 0.00	0.05	0.10	1.26	1 27
1 027	0.34		(a) 0.00	0.04	- 0.06	-0.38	-0.37
L029	0.36	(a) $(c)$ 0.08	(b) 0.00	- 0.04	0.00	0.56	0.57
L030	0.16	(u) (c) 0.00		-	-	-0.85	-0.84
L031	0.26		(a) 0.09	-	0.10	-0.01	0.00
L033	0.31		0.11	0.01	0.12	0.30	0.31
L034	0.25	(a) 37.00		-	-	-0.07	-0.07
L035	0.13	(c) 0.03		0.01	0.10	-1.21	-1.20
L036	0.77		0.04	-	-	1.88	1.89
L037	0.26		0.08	0.10	0.20	-0.01	0.00
L038	0.28		(b) 0.02	0.05	0.10	0.12	0.13
L040	0.24		0.05	0.02	0.05	-0.15	-0.14
L041	0.20	(c) 0.06		0.02	0.04	-0.46	-0.46
L042	0.20	21.44		-	-	-0.46	-0.46
L044	0.17	(D) (C) 0.04	0.02	0.05	0.10	-0.74	-0.74
1 047	0.32	(a) (c) 0.07	0.05	0.05	0.10	-0.33	-0.29
1 050	0.22	(a) (c) 0.07 (a) (f) 159.55		0.05	0.10	0.50	0.23
L051	0.25	(u) (i) 100.00	(a) 0.09	0.05	0.10	-0.07	-0.07
L055	0.55	(c) 0.07	(4) 0100	0.01	0.05	1.30	1.30
L056	0.21	(0) 0101	0.08	< 0.10	0.10	-0.39	-0.39
L058	0.32		(b) 0.23	0.01	0.05	0.35	0.36
L059	0.33		(b) 0.24	0.01	0.05	0.41	0.41
L060	0.24	(c) 0.09		-	-	-0.15	-0.14
L061	0.20	18.60		0.01	0.04	-0.46	-0.46
L062	0.23	(a) 26.00		-	-	-0.22	-0.21
L063	0.26	<u>.</u>	0.24	< 0.04	< 0.04	-0.01	0.00
L069	0.29	33.88		0.01	0.03	0.18	0.19
L070	0.20	14.30	0.05	-	0.01	-0.46	-0.46
LU71	0.15		0.05	0.001	0.03	-0.96	-0.96
L073	0.00	25.00	(a) 0.00	-	- 0.10	-2.07	-2.00
1077	0.23	(c) 0.10		0.03	0.10	-0.22	-0.21
1 078	0.23	(c) 0.10 (a) (c) 0.25		- 0.01	- 0.08	1 42	1 42
L079	0.20	(u) (c) 0.20 9.60		0.01	0.04	-0.46	-0.46
L081	0.33	0100	(a) 0.05	-	-	0.41	0.41
L082	0.23		0.06	0.01	0.10	-0.22	-0.21
L083	0.16		(a) 0.06	0.01	0.05	-0.85	-0.84
L085	0.18		0.05	0.02	0.09	-0.64	-0.64
L087	0.59		(b) 0.00	-	-	1.42	1.42
L088	0.46	(a) 16.50		0.01	0.05	0.99	0.99
L096	0.38		(b) 0.05	0.02	0.20	0.65	0.66
L097	0.21	49.99		0.04	0.10	-0.38	-0.37
L100	0.31		0.24	0.05	0.10	0.30	0.31
1101	0.20		0.03	-	-	-0.46	-0.46
L105	0.24	(a) 0.00		-	-	-0.15	-0.14

**Table 5:** z-scores for event GA21 maize powder level 2 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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, (e) U seems to be underestimated. Uncertainties were sorted according to laboratory number. Results are as submitted by participants.

		Ma	aize e	vent	GA21	/m 9/		
Laboratory			Assian	st mea	an = 1.92 m lue = 2.08 i	/m % n/m %		
number	Value	Uncerta	inty		LOD m/m	LOQ m/m	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	abs	olute				
L002	0.84		(a)	0.06	-	-	-1.44	-1.58
L003	1.35			0.65	0.04	0.10	-0.61	-0.75
L004	2.18	(c) 0.65		4 00	-	-	0.22	0.08
1005	1 22		(b)	0.20	0.05	0.20	-0.79	-0 93
L000	2.32		(b)	0.20	< 0.10	0.10	0.33	0.19
L008	6.38		(a)	3.06	-	-	2.08	1.95
L009	7.64	23.57			-	-	2.40	2.26
L011	1.37		(a)	0.67	0.01	0.10	-0.59	-0.73
L012	0.32		(a)	0.00	0.10	0.10	-0.13	-0.27
L015	1.48	(a) (c) 0.44		0.75	0.02	0.05	-0.45	-0.59
L016	1.67	(4) (6) 6111	(a)	0.46	0.10	0.99	-0.24	-0.38
L017	1.69	70.00	. ,		0.08	0.08	-0.22	-0.36
L018	1.56		(a)	0.77	-	-	-0.36	-0.50
L019	1.29			0.63	-	-	-0.69	-0.83
L020	1.23	49.00		0.32	0.01	0.10	-0.78	-0.91
L023	1.76	10.00		0.55	0.03	0.10	-0.15	-0.29
L024	0.88		(a)	0.00	-	-	-1.36	-1.49
L025	4.51	(c) 0.62			0.05	0.10	1.48	1.34
L026	3.79		(a)	0.00	-	-	1.18	1.04
LU27	1.59	(2) $(2)$ 0 40	(D)	0.26	0.04	0.06	-0.33	-0.47
L030	1.82	(a) (c) 0.40					-0.10	-0.23
L031	2.53		(a)	0.87	-	0.10	0.48	0.34
L033	2.12		. ,	0.29	0.01	0.12	0.17	0.03
L034	1.70	(a) (e) 5.00			-	-	-0.21	-0.35
L035	1.68	(c) 0.32		2 62	0.01	0.10	-0.23	-0.37
L036 L037	9.07 2.11			3.62 0.24	- 0 10	0.20	2.04	0.02
L038	1.82		(b) (e)	0.10	0.05	0.10	-0.10	-0.23
L040	1.92			0.40	0.02	0.05	0.00	-0.14
L041	1.23	(c) 0.21			0.02	0.04	-0.78	-0.91
L042	1.60	21.44 (b) (c) 0.17			-	-	-0.32	-0.46
L044	2.76	(b) (c) 0.17	(e)	0 12	0.05	0.10	-0.15	-0.29
L047	1.60	(a) (c) 0.40	(0)	0.12	0.05	0.10	-0.32	-0.46
L050	6.90	(a) 17.82			0.05	0.10	2.22	2.08
L051	1.83		(a)	0.36	0.05	0.10	-0.09	-0.22
L055	3.43	(c) 1.00		0.40	0.01	0.05	1.01	0.87
1.058	1.41		(b)	0.18	< 0.10	0.10	-0.54	-0.00
L059	2.63		(b)	1.66	0.01	0.03	0.54	0.41
L060	1.50	(c) 0.18	(-)		-	-	-0.43	-0.57
L061	1.60	11.40			0.01	0.04	-0.32	-0.46
L062	1.64	(a) 26.00		0.50	-	-	-0.28	-0.41
L063	1.49	27 55		0.50	< 0.04	< 0.04	-0.44	-0.58
L009	1.50	11 60			0.01	0.03	-0.43	-0.57
L071	1.90			0.60	0.001	0.05	-0.02	-0.16
L073	0.01		(a)	0.15	-	-	-9.14	-9.27
L076	1.71	25.00			0.03	0.10	-0.20	-0.34
L077	1.58	(c) 0.40			-	-	-0.34	-0.48
	1 80	(a)(c)(e) 0.04 7 50			< 0.01	< 0.08 0.04	-0.11	-0.25
L081	2.79	7.00	(a)	0.50	- 0.01	- 0.04	0.65	0.51
L082	1.53		()	0.26	0.01	0.10	-0.40	-0.53
L083	1.33		(a)	0.40	0.01	0.05	-0.64	-0.78
L085	1.54			0.38	0.02	0.09	-0.39	-0.52
L087	5.77 2 FF	-		-	-	-	1.91	1.77
L000	2.55	(a) 16.50	(b)	0 75	0.01	0.05	0.49	0.35
L097	1.91	33.14	(0)	0.70	0.02	0.20	-0.01	-0.15
L100	2.18	· ·		0.57	0.05	0.10	0.22	0.08
L101	1.80			0.10	-	-	-0.11	-0.25
L105	1.78	-		-	-	-	-0.13	-0.27

**Table 6**: z-scores for event GA21 maize powder level 1 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> 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. Results are as submitted by participants.

		Μ	aize ever	nt GA21			
			Robust me	ean = <i>0.31 c</i>	o/cp %		
Laboratory			Assigned v	alue = 0.14 d	cp/cp %		
number	Value	Uncerta	inty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L005	0.67		0.13	-	-	1.36	2.67
L009	0.47	2.77		-	-	0.75	2.06
L010	0.50	(c) 0.10		-	-	0.86	2.16
L024	< 0.10		(a) 0.00	0.01	0.10	*	*
L026	0.54		(a) 0.12	-	-	0.99	2.30
L028	< 0.10		-	0.05	0.10	*	*
L029	0.32	(a) (c) 0.08		-	-	0.08	1.39
L030	0.08	(c) 0.04		0.01	0.10	-2.33	-1.02
L032	0.10	32.00		0.05	0.10	-1.94	-0.63
L039	0.13	(b) 0.29		0.10	0.10	-1.48	-0.18
L043	0.23	(a) 49.40		-	-	-0.49	0.82
L046	1.50	10.75		-	-	2.76	4.07
L049	0.20	(c) 0.05		0.001	0.01	-0.74	0.57
L052	1.06		-	-	-	2.16	3.47
L054	0.21		0.06	0.05	0.10	-0.65	0.66
L060	0.12	(c) 0.07		-	-	-1.62	-0.31
L065	1.50	(a) 25.00		-	-	2.76	4.07
L066	0.06		(a) 0.06	-	-	-2.98	-1.67
L067	0.50		0.15	0.04	0.06	0.86	2.16
L068	5.09		(a) 0.74	-	-	4.89	6.20
L074	0.16	38.00		-	-	-1.12	0.19
L080	0.30	-		-	-	-0.03	1.28
L083	0.16		(a) 0.06	-	-	-1.12	0.19
L089	1.14	(a) 25.00		-	-	2.29	3.60
L095	0.05	-		0.03	0.05	-3.14	-1.84
L098	> 0.01	-		0.01	-	*	*
L099	0.25		(a) 0.15	-	-	-0.35	0.96

**Table 7**: z-scores for event GA21 maize powder level 2 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> 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 underestimated. Results are as submitted by participants.

		Ма	aize ever	nt GA21						
			Robust me	ean = <i>1.</i> 76 cµ	o/ср %					
Laboratory			Assigned value = 0.86 cp/cp %							
number	Value	Uncertai	nty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>			
		relative	absolute							
L005	5.10		1.39	-	-	1.85	3.10			
L009	3.82	23.57		-	-	1.35	2.60			
L010	5.00	(c) 0.60		-	-	1.81	3.07			
L024	0.44		(a) 0.00	0.01	0.10	-2.41	-1.16			
L026	3.79		(a) 1.24	-	-	1.33	2.59			
L028	0.70		0.38	0.05	0.10	-1.60	-0.35			
L029	1.81	(a) (c) 0.43		-	-	0.05	1.30			
L030	0.91	(c) 0.18		0.01	0.10	-1.14	0.11			
L032	0.80	32.00		0.05	0.10	-1.37	-0.12			
L039	2.07	(b) 0.29		0.10	0.10	0.28	1.53			
L043	1.88	(a) 49.40		-	-	0.12	1.37			
L046	0.27	23.48		-	-	-3.25	-2.00			
L049	1.31	(c) 0.42		0.001	0.01	-0.51	0.74			
L052	4.96		-	-	-	1.80	3.05			
L054	2.11		0.80	0.05	0.10	0.32	1.57			
L060	0.75	(c) 0.15		-	-	-1.48	-0.23			
L065	7.60	(a) 25.00		-	-	2.54	3.79			
L066	0.36		(a) 0.07	-	-	-2.74	-1.49			
L067	3.60		0.50	0.04	0.06	1.24	2.50			
L068	8.41		(a) 3.12	-	-	2.72	3.97			
L074	0.95	(e) 3.20		-	-	-1.07	0.18			
L080	1.70	-		-	-	-0.06	1.19			
L083	1.33		(a) 0.40	-	-	-0.49	0.77			
L089	12.28	(a) 25.00		-	-	3.38	4.63			
L095	0.38	-		0.03	0.05	-2.66	-1.41			
L098	> 0.01	-		0.01	-	*	*			
L099	1.88	(a) 1.13		-	-	0.12	1.37			

**Table 8**: z-scores for event TC1507 maize powder level 1 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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, (e) *U* seems to be underestimated. Results are as submitted by participants.

		Mai	ze event	TC1507			
Laboratory			Robust mea	an = 0.38 n	n/m % m/m %		
number	Value	Uncerta	ainty	LOD m/m	LOQ m/m	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L001	0.38		(a) 0.10	0.10	0.10	0.01	0.51
L002	0.34		(a) 0.03	-	- 0.10	-0.23	0.27
L003	0.58	(c) 0.16	0.17	0.04	0.10	0.01	1.24
L005	0.44	(0) 0110	0.09	0.08	0.30	0.33	0.83
L006	0.63		(b) 0.34	-	-	1.11	1.61
L007	0.80		(b) 0.40	< 0.10	0.10	1.63	2.13
	0.40	(e) 1.88	(a) 0.16	-	-	0.13 4 04	4 53
L011	0.52	(0) 1100	(a) 0.20	0.01	0.10	0.70	1.19
L012	0.75		(a) 0.00	0.10	0.10	1.49	1.99
L013	0.48	(-) 0.11	0.22	0.02	0.08	0.52	1.02
L015	0.30	(C) 0.11 40.00		0.02	0.05	-0.10	0.40
L018	0.37	10.00	(a) 0.10	-	-	-0.04	0.46
L019	0.45		0.13	-	-	0.38	0.88
L020	0.29	44.00	0.04	0.01	0.10	-0.57	-0.07
LU21	0.44	41.00		-		0.33	0.83
L023	0.39		0.08	0.02	0.10	0.07	0.57
L024	0.24		(a) 0.00	0.01	0.10	-0.98	-0.48
L025	0.18	(c) 0.01		0.10	0.10	-1.61	-1.11
L026	0.40		(a) 0.00 (b) 0.09	-		0.13	0.62
L029	0.66	(a) (c) 0.28	(b) 0.03	-	-	1.21	1.71
L030	0.32	-		-	-	-0.36	0.14
L031	0.50		(a) 0.12		0.10	0.61	1.11
L033	0.37	(2) 20.00	0.07	0.05	0.12	-0.04	0.46
L034	0.32	(c) 0.13		0.01	0.10	0.01	0.14
L036	0.22	(0) 0110	0.05	-	-	-1.17	-0.67
L037	0.43		0.12	0.10	0.20	0.28	0.78
L038	0.38		(b) 0.04	0.03	0.10	0.01	0.51
L040	0.33	(c) 0.11	0.00	0.02	0.03	-0.29	0.21
L042	0.20	(e) 5.40		-	-	-1.38	-0.88
L044	0.45	(b) (c) 0.09		0.04	0.08	0.38	0.88
L045	0.64		0.03	-	- 0.10	1.13	1.63
L047	0.23	(a) (c) 0.07 47.68		0.04	0.10	-1.00	-0.56
L051	0.46		(a) 0.12	0.05	0.10	0.43	0.93
L055	4.18	(e) 1.23		0.01	0.05	5.22	5.72
L056	0.33		0.02	< 0.10	0.10	-0.28	0.21
L058 L059	0.33		(b) 0.17 (b) 0.15	0.01	0.05	-0.29	0.21
L060	0.40	(c) 0.12	(5) 0.10	-	-	0.13	0.62
L061	0.30	16.70		0.01	0.04	-0.50	0.00
L062	0.36	(a) 50.00		< 0.05	0.10	-0.10	0.40
	0.31	9.59 18 30		0.12	0.35	-0.43	0.07
L071	0.30	10.00	0.10	0.001	0.10	-0.50	0.00
L073	0.10		(a) 0.00	-	-	-2.88	-2.39
L077	0.29	(c) 0.10		-	-	-0.57	-0.07
L078	0.43	(a) (c) 0.27		< 0.01	< 0.10	0.28	0.78
L081	0.50	10.10	(a) 0.16		- 0.04	0.61	1.11
L082	0.57		0.13	0.01	0.04	0.90	1.39
L083	0.17		(a) 0.05	0.01	0.05	-1.73	-1.23
LU85 I 087	0.28		0.07 (h) 0.00	0.01	0.05	-0.65	-0.15
L096	0.45		(b) 0.00 (b) 0.07	0.02	0.20	0.38	0.88
L097	0.75	27.66		0.04	0.10	1.49	1.99
L100	0.43		-	0.05	0.10	0.28	0.78
L101	0.45		0.04	-	-	0.38	0.88

**Table 9**: z-scores for event TC1507 maize powder level 2 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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, (e) Useems to be underestimated. Results are as submitted by participants.

Robust mean = 1.07 m/m %           Assigned value = 0.89 m/m %           LoDo 100         c.score <sup>3</sup> z.score <sup>3</sup> L001         0.94         (a) 0.30         0.10         0.10         -0.27         0.12           L003         0.32         0.37         0.04         0.10         -0.35         0.93           L006         0.44         (b) 0.10         -         -         -0.27         0.12           L007         1.99         (b) 0.10         -         -         -0.01         0.38           L008         1.06         -         -         4.30         4.66           L011         1.45         (a) 0.55         0.01         0.10         0.15         0.12           L013         1.49         0.06         0.02         0.08         0.03         0.10         0.10         0.22         0.61           L017         1.18         40.00         0.10         0.10         0.22         0.61            0.88         (a) 0.26			Mai	ze event 7	FC1507			
Laboratory number         Assigned value = 0.89 m/m ½         z-score         z-score         z-score           1001         0.94         (a) 0.30         0.10         0.10         0.00         0.01         0.00         0.02         0.11           1002         0.95         (a) 0.30         0.37         0.04         0.10         0.02         0.01           1004         1.38         (c) 0.41         0.39         0.08         0.30         0.14         0.35           1005         1.14         0.39         0.08         0.30         0.14         0.38           1006         0.44         (b) 0.10         -         -         -0.27         0.12           1007         1.09         (b) 0.10         <0.10         0.10         0.05         0.44           1008         (a) 0.46         -         -         0.40         4.83           1009         7.72         10.86         (a) 0.00         0.10         0.10         1.52         1.92           1011         1.45         (a) 0.00         0.10         0.10         0.12         1.92           1013         1.49         0.66         0.02         0.05         0.44           1021 <th></th> <th></th> <th></th> <th>Robust mea</th> <th>an = 1.07 n</th> <th>n/m %</th> <th></th> <th></th>				Robust mea	an = 1.07 n	n/m %		
Number         Value         Uncertainty         LOD num Log num         2+score         2+score	Laboratory			Assigned val	lue = 0.89	m/m %	1	2
	number	value	Uncer	absolute	LOD m/m	LOQ m/m	z-score	z-score <sup>-</sup>
L002         0.95         (a) 0.05         -         -         -0.25         0.07           L004         1.38         (c) 0.41         0.39         0.08         0.30         0.14         0.55           L005         1.14         0.39         0.08         0.30         0.14         0.55           L006         0.24         (b) 0.10         -         -         -0.27         0.12           L007         1.09         (b) 0.10         <0.10         0.10         0.05         0.44           L008         1.06         (a) 0.55         0.01         0.10         0.55         0.05           L011         1.45         (a) 0.05         0.01         0.10         0.57         1.22           L013         1.49         0.66         0.02         0.08         0.73         1.12           L017         1.18         40.00         0.10         0.10         0.22         0.61           L018         0.88         (a) 0.25         -         -         0.42         0.61           L021         1.57         26.00         -         -         0.84         1.23           L024         0.68         (a) 0.00         0.10         <	L001	0.94	Tolativo	(a) 0.30	0.10	0.10	-0.27	0.12
	L002	0.95		(a) 0.05	-	-	-0.25	0.14
	L003	0.92	(-) 0.44	0.37	0.04	0.10	-0.32	0.07
L006         0.34         (b)         0.05         0.05         0.05         0.07         0.12         0.07           L007         1.09         (b)         0.10         <0.10         0.10         0.05         0.44           L008         1.06         (a)         0.46         -         -         4.30         4.89           L011         1.45         (a)         0.55         0.01         0.10         0.157         1.92           L013         1.49         0.66         0.02         0.08         0.73         1.12           L015         1.09         (c)         0.33         0.02         0.055         0.44           L017         1.18         40.00         0.10         0.10         0.22         0.61           L018         0.88         0.21         -         -         -0.77         0.37           L021         1.57         26.00         -         -         0.84         1.23           L021         1.57         26.00         -         -         0.03         0.42           L027         1.00         (b)         0.23         -         -         0.14         0.25           L024	L004	1.38	(C) 0.41	0.39	- 0.08	0.30	0.56	0.95
L007         1.09         (b) 0.10         < 0.10	L006	0.94		(b) 0.10	-	-	-0.27	0.12
	L007	1.09		(b) 0.10	< 0.10	0.10	0.05	0.44
	L008	1.06		(a) 0.46	-	-	-0.01	0.38
LD11         1.43         (a) 0.55         0.01         0.10         0.07         1.10           L012         2.15         (a) 0.00         0.10         0.10         0.152         1.92           L013         1.49         0.66         0.02         0.08         0.73         1.12           L015         1.09         (c) 0.33         0.02         0.05         0.44           L017         1.18         40.00         0.10         0.10         0.22         0.61           L018         0.88         (a) 0.25         -         -         -0.42         0.02           L020         0.94         0.14         0.01         0.10         -0.27         0.37           L020         0.94         0.14         0.01         0.10         -0.24         0.63           L021         1.57         26.00         0.10         0.10         -0.24         0.63           L023         1.74         (a) (c) 0.75         -         -         0.03         0.42           L025         0.75         (c) 0.15         0.01         0.10         0.22         0.61           L033         0.96         0.22         0.05         0.12         -614	L009	7.72	10.86		-	-	4.30	4.69
Lora         Lins         (a)         0.05         0.10         0.10         0.02         0.05         0.073         1.12           Loff         1.09         (c)         0.33         0.02         0.05         0.05         0.44           Loff         1.18         40.00         0.10         0.10         0.22         0.61           Loff         0.75         0.21         -         -         -0.42         -0.02           Lo20         0.94         0.14         0.01         0.10         0.10         -27         0.12           Lo21         1.57         26.00         -         -         0.84         1.23           Lo22         0.75         (c)         0.09         0.10         0.10         -0.77         0.37           Lo25         0.75         (c)         0.09         -         -         0.03         0.42           Lo27         1.00         (b)         0.23         -         -         -         0.16         1.46           L030         0.98         -         -         -         0.18         0.21         -         -         0.33         0.10           L033         0.90         (c) </th <th>L011</th> <th>1.45</th> <th></th> <th>(a) 0.55 (a) 0.00</th> <th>0.01</th> <th>0.10</th> <th>0.67</th> <th>1.00</th>	L011	1.45		(a) 0.55 (a) 0.00	0.01	0.10	0.67	1.00
L015         1.09         (c) 0.33         0.02         0.05         0.05         0.44           L017         1.18         40.00         0.10         0.10         0.10         0.22         0.02           L019         0.75         0.21         -         -         -0.77         0.37           L020         0.94         0.14         0.01         0.10         -0.27         0.12           L021         1.57         26.00         -         -         0.84         1.23           L023         1.19         0.26         0.02         0.10         0.44         1.23           L024         0.68         (a) 0.00         0.10         0.10         -0.77         -0.37           L025         0.75         (c) 0.075         -         -         0.14         0.22           L037         1.00         (b) 0.23         -         -         -0.14         0.25           L032         1.74         (a) 0.26         0.02         0.05         0.12         -0.23           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L033         0.90         (c) 0.15         0.01	L012	1.49		(a) 0.00 0.66	0.10	0.10	0.73	1.12
L017         1.18         40.00         0.10         0.10         0.22         0.61           L018         0.88         (a) 0.25         -         -         -0.42         -0.02           L020         0.94         0.14         0.01         -0.27         0.12           L021         1.57         26.00         -         -         0.24         0.63           L023         1.19         0.26         0.02         0.10         0.24         0.63           L024         0.68         (a) 0.00         -         -         0.03         0.42           L025         0.75         (c) 0.09         0.10         0.10         -0.77         -0.37           L026         1.08         (a) (c) 0.75         -         -         1.06         1.46           L030         0.98         -         -         -         -0.18         0.21           L031         1.18         (a) 0.22         0.05         0.12         -0.23         0.16           L033         0.39         (a) 19.00         -         -         -         0.30         0.10           L034         0.33         (a) 19.00         -         -         -	L015	1.09	(c) 0.33		0.02	0.05	0.05	0.44
L018         0.88         (a)         0.25         -         -         -0.77         -0.37           L020         0.94         0.14         0.01         0.10         -0.27         -0.37           L021         1.57         26.00         -         -         0.84         1.23           L023         1.19         0.26         0.02         0.10         0.24         0.68           L025         0.75         (c)         0.09         0.10         0.10         -0.77         -0.37           L026         1.08         (a)         0.00         -         -         0.03         0.42           L027         1.00         (b)         0.23         -         -         -         0.14         0.25           L030         0.98         -         -         -         -         0.14         0.22         0.61           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L033         0.90         (c)         0.15         0.01         0.10         0.22         0.61           L034         0.93         (a)         1.90         -         -         -         0.30	L017	1.18	40.00		0.10	0.10	0.22	0.61
L019         0.75         0.21         -         -         -         -0.77         -0.37           L021         1.57         26.00         -         -         0.84         1.23           L023         1.19         0.26         0.02         0.10         0.24         0.63           L025         0.75         (c) 0.09         0.10         0.10         -0.77         -0.37           L026         1.08         (a) 0.00         -         -         0.03         0.42           L027         1.00         (b) 0.23         -         -         0.14         0.25           L029         1.74         (a) (c) 0.75         -         -         1.06         1.46           L031         1.18         (a) 0.28         0.10         0.22         0.65           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L033         0.90         (c) 0.15         0.01         0.10         0.20         -0.88         0.22           L036         0.82         0.14         0.10         0.20         -0.8         0.21           L033         0.90         (c) 0.24         0.01         0.02	L018	0.88		(a) 0.25	-	-	-0.42	-0.02
	L019	0.75		0.21	-	- 0.10	-0.77	-0.37
L023         1.19         0.26         0.02         0.10         0.24         0.68           L024         0.68         (a) 0.00         0.01         0.10         -0.98         -0.58           L025         0.75         (c) 0.09         0.10         0.10         -0.77         -0.37           L026         1.08         (a) 0.00         -         -         0.03         0.42           L027         1.00         (b) 0.23         -         -         -0.14         0.22           L030         0.98         -         -         -         -         -0.18         0.21           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L034         0.93         (a) 19.00         -         -         -0.30         0.10           L035         0.90         (c) 0.15         0.01         0.10         0.22         0.61           L034         0.32         0.13         0.10         0.22         0.61           L044         0.98         (c) 0.24         0.01         0.02         -0.18           L044         0.33         (b) (c) 0.07         0.04         0.08         0.48 </th <th>L020</th> <th>0.94</th> <th>26.00</th> <th>0.14</th> <th>0.01</th> <th>0.10</th> <th>-0.27</th> <th>1.23</th>	L020	0.94	26.00	0.14	0.01	0.10	-0.27	1.23
L024         0.68         (a) 0.00         0.01         0.10         -0.98         -0.58           L025         0.75         (c) 0.09         0.10         0.10         0.177         -0.37           L026         1.08         (a) 0.00         -         -         0.03         0.42           L027         1.00         (b) 0.23         -         -         -0.14         0.25           L030         0.98         -         -         -         -0.16         1.46           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L034         0.93         (a) 19.00         -         -         -0.73         0.02           L036         0.82         0.18         0.10         0.10         -0.23         0.16           L035         0.90         (c) 0.15         0.01         0.02         -0.08         0.32           L036         0.82         0.18         0.10         0.02         -0.18         0.21           L040         0.94         0.15         0.02         0.05         -         1.67         2.06           L044         0.33         (b) 0.60         0.01	L023	1.19	20.00	0.26	0.02	0.10	0.24	0.63
L025         0.75         (c) 0.09         0.10         0.10         0.10         -0.77         0.03         0.42           L027         1.00         (b) 0.23         -         -         -0.14         0.25           L029         1.74         (a) (c) 0.75         -         -         -1.06         1.46           L031         0.98         -         -         -         -         -0.13         0.21           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L034         0.93         (a) 19.00         -         -         -         -0.57         -0.18           L035         0.90         (c) 0.15         0.011         0.10         -0.37         0.02           L036         0.82         0.18         -         -         -0.57         -0.18           L037         1.03         0.10         0.10         0.22         0.61         L044         0.32         0.61           L044         0.34         (b) 0.67         0.04         0.08         0.48         0.87           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87	L024	0.68		(a) 0.00	0.01	0.10	-0.98	-0.58
L026         1.08         (a) 0.00         -         -         0.03         0.42           L027         1.00         (b) 0.23         -         -         0.14         0.25           L029         1.74         (a) (c) 0.75         -         -         0.166         1.46           L030         0.98         -         -         -         -         0.18         0.21           L031         1.18         (a) 0.28         0.10         0.12         -0.23         0.16           L033         0.90         (c) 0.15         0.01         0.10         0.10         0.22         0.61           L035         0.90         (c) 0.15         0.01         0.10         0.20         -0.08         0.32           L036         0.82         0.18         -         -         -0.57         -0.18           L041         0.94         0.15         0.02         0.05         -0.27         0.12           L044         0.94         0.15         0.02         0.05         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L044         1.33         (b) (c	L025	0.75	(c) 0.09		0.10	0.10	-0.77	-0.37
L027         1.00         (b)         0.23         -         0.18         0.21         0.23         0.16         0.23         0.16         0.23         0.16         0.23         0.16         0.23         0.16         0.23         0.16         0.23         0.16         0.23         0.16         0.33         0.10         0.12         0.033         0.10         0.10         0.20         0.08         0.32         0.03         0.10         0.10         0.20         -0.08         0.32         0.03         0.10         0.22         0.61         1.33         0.02         -0.07         0.04         0.02         -0.08         0.32         0.03         0.10         0.22         0.01         0.22         0.61         1.33         0.02         0.03         0.10         0.14         0.33         0.10         0.14         0.33         0.10         0.14         0.31         1.00         0.01         0.025	L026	1.08		(a) 0.00	-	-	0.03	0.42
	L027	1.00	(2) $(2)$ 0.75	(b) 0.23	-	-	-0.14	0.25
L031         1.18         (a) 0.28         0.10         0.22         0.61           L033         0.96         0.22         0.05         0.12         -0.23         0.16           L034         0.93         (a) 19.00         -         -         -         -0.30         0.10           L035         0.90         (c) 0.15         0.01         0.10         -0.37         0.02           L036         0.82         0.18         -         -         -0.57         -0.18           L037         1.03         0.10         0.10         0.22         0.61         0.22         0.61           L040         0.94         0.01         0.02         -0.08         0.32         0.10         0.22         0.61           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21         L042         2.30         (e) 5.40         -         -         1.67         2.06           L042         2.30         (e) 5.40         -         -         1.67         2.06         1.05         0.22         0.61         0.02         0.44         0.80         0.80         0.80         0.21         0.11         0.16         0.55	L030	0.98	(a) (c) 0.75				-0.18	0.21
L033         0.96         0.22         0.05         0.12         -0.23         0.16           L034         0.93         (a) 19.00         -         -         -         -0.30         0.10           L035         0.90         (c) 0.15         0.01         0.10         -0.37         0.02           L036         0.82         0.18         -         -         -0.57         -0.18           L037         1.03         0.10         0.10         0.20         -0.08         0.32           L038         1.18         (b) 0.06         0.03         0.10         0.22         0.61           L040         0.94         0.15         0.02         0.05         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L042         2.30         (e) 5.40         -         -         1.67         2.06           L044         1.33         (b) (c) 0.07         0.04         0.01         0.02         -0.18         0.21           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20	L031	1.18		(a) 0.28		0.10	0.22	0.61
L034         0.93         (a) 19.00         -         -         -         -0.30         0.10           L035         0.90         (c) 0.15         0.01         0.01         -0.37         0.02           L036         0.82         0.18         -         -         -0.57         -0.18           L037         1.03         0.10         0.20         -0.08         0.32           L038         1.18         (b) 0.06         0.03         0.10         0.22         0.61           L040         0.94         0.15         0.02         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20         0.04         0.10         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         0.20<	L033	0.96		0.22	0.05	0.12	-0.23	0.16
L035         0.90         (c) 0.15         0.01         0.01         -0.37         0.02           L036         0.82         0.18         -         -         -0.57         -0.18           L037         1.03         0.10         0.10         0.20         -0.08         0.32           L038         1.18         (b) 0.06         0.03         0.10         0.22         0.61           L040         0.94         0.15         0.02         0.05         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L045         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.16         0.56           L055         18.90         (e) 3.04         0.01         0.05         0.20         0.59           L055         18.90         (b) 0.57         0.01         0	L034	0.93	(a) 19.00		-	-	-0.30	0.10
L036         0.82         0.18         -         -         -         -0.17         -0.18           L037         1.03         0.10         0.10         0.20         -0.08         0.32           L038         1.18         (b) 0.06         0.03         0.10         0.22         0.61           L040         0.94         0.15         0.02         0.05         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L042         2.30         (e) 5.40         -         -         1.67         2.06           L045         1.95         0.28         -         -         1.31         1.70           L045         1.95         0.20         0.04         0.10         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         0.20         0.59           L055         1.15         0.24         <0.10 <td< th=""><th>L035</th><th>0.90</th><th>(c) 0.15</th><th>0.40</th><th>0.01</th><th>0.10</th><th>-0.37</th><th>0.02</th></td<>	L035	0.90	(c) 0.15	0.40	0.01	0.10	-0.37	0.02
L037         1.135         0.105         0.105         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.105         0.102         0.011         0.022         0.011         0.022         0.011         0.022         0.011         0.022         0.011         0.022         0.011         0.022         0.013         0.101         0.022         0.013         0.101         0.022         0.013         0.101         0.012         0.011         0.021         0.011         0.021         0.011         0.011         0.011         0.015         0.221         0.011         0.011         0.015         0.211         0.114         0.011         0.015         0.221         0.011         0.111	L036	0.82		0.18	- 0.10	- 0.20	-0.57	-0.18
L040         0.94         0.15         0.02         0.05         -0.27         0.12           L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L042         2.30         (e) 5.40         -         -         -         1.67         2.06           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20         0.04         0.10         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         -1.47         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         -0.10         0.10         0.16         0.56           L058         1.17         (b) 0.57         0.01         0.05         -0.23         0.16           L061         0.90         10.60	L038	1.18		(b) 0.06	0.03	0.10	0.22	0.61
L041         0.98         (c) 0.24         0.01         0.02         -0.18         0.21           L042         2.30         (e) 5.40         -         -         1.67         2.06           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20         0.04         0.01         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         <0.10	L040	0.94		0.15	0.02	0.05	-0.27	0.12
L042         2.30         (e) 5.40         -         -         -         1.67         2.06           L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20         0.04         0.10         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.24         <0.01	L041	0.98	(c) 0.24		0.01	0.02	-0.18	0.21
L044         1.33         (b) (c) 0.07         0.04         0.08         0.48         0.87           L045         1.95         0.28         -         -         1.31         1.70           L047         0.79         (a) (c) 0.20         0.04         0.10         -0.65         -0.26           L050         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         <0.10         0.10         0.16         0.56           L058         1.17         (b) 0.57         0.01         0.05         0.20         0.59           L060         1.10         (c) 0.17         -         -         0.07         0.46           L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a) 50.00         <0.05         0.10         0.12         0.35           L069         0.96         39.66         0.12	L042	2.30	(e) 5.40		-	-	1.67	2.06
L047       0.79       (a) (c) 0.20       0.04       0.10       -0.65       -0.26         L050       0.50       53.11       0.05       0.10       -1.67       -1.27         L051       1.14       (a) 0.24       0.05       0.10       0.14       0.56         L055       18.90       (e) 3.04       0.01       0.05       6.24       6.64         L056       1.15       0.24       <0.10       0.10       0.16       0.56         L058       1.17       (b) 0.57       0.01       0.05       0.20       0.59         L059       0.96       (b) 0.47       0.01       0.05       -0.23       0.16         L060       1.10       (c) 0.17       -       -       0.07       0.46         L061       0.90       10.60       0.01       0.04       -0.37       0.02         L062       1.13       (a) 50.00       <0.05       0.10       0.12       0.52         L069       0.96       39.66       0.12       0.35       -0.23       0.16         L070       1.35       15.40       -       0.01       0.51       0.90         L071       0.90       0.30       0.001 <th>L044</th> <th>1.33</th> <th>(b) (c) 0.07</th> <th>0.28</th> <th>0.04</th> <th>80.0</th> <th>0.48</th> <th>0.87</th>	L044	1.33	(b) (c) 0.07	0.28	0.04	80.0	0.48	0.87
L050         0.50         53.11         0.05         0.10         -1.67         -1.27           L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         < 0.10         0.16         0.56           L058         1.17         (b) 0.57         0.01         0.05         0.20         0.59           L059         0.96         (b) 0.47         0.01         0.05         -0.23         0.16           L060         1.10         (c) 0.17         -         -         0.07         0.46           L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a) 50.00         <0.05         0.10         0.12         0.52           L069         0.96         39.66         0.12         0.35         -0.23         0.16           L070         1.35         15.40         -         0.01         0.51         0.90           L071         0.90         0.30         0.001         0.04         -0	L045	0.79	(a) (c) 0.20	0.20	0.04	0.10	-0.65	-0.26
L051         1.14         (a) 0.24         0.05         0.10         0.14         0.54           L055         18.90         (e) 3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         < 0.10	L050	0.50	53.11		0.05	0.10	-1.67	-1.27
L055         18.90         (e)         3.04         0.01         0.05         6.24         6.64           L056         1.15         0.24         < 0.10         0.10         0.16         0.56           L058         1.17         (b)         0.57         0.01         0.05         0.20         0.59           L059         0.96         (b)         0.47         0.01         0.05         -0.23         0.16           L060         1.10         (c)         0.17         -         -         0.07         0.46           L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a)         50.00         <0.05         0.10         0.12         0.52           L069         0.96         39.66         0.12         0.35         -0.23         0.16           L070         1.35         15.40         -         0.01         0.51         0.90           L071         0.90         0.30         0.001         0.04         -0.63         -1.03           L073         0.25         (a)         0.15         -         -         -3.15         -2.76	L051	1.14		(a) 0.24	0.05	0.10	0.14	0.54
L056         1.15         0.24         < 0.10	L055	18.90	(e) 3.04		0.01	0.05	6.24	6.64
L055         1.17         (b)         0.57         0.01         0.05         0.20         0.39           L059         0.96         (b)         0.47         0.01         0.05         -0.23         0.16           L060         1.10         (c)         0.17         -         -         0.07         0.46           L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a)         50.00         <0.05	L056	1.15		0.24	< 0.10	0.10	0.16	0.56
L060         1.10         (c)         0.11         0.00         0.00         0.01         0.01         0.02         0.07         0.46           L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a) 50.00         < 0.05	1 059	0.96		(b) 0.57 (b) 0.47	0.01	0.05	-0.23	0.59
L061         0.90         10.60         0.01         0.04         -0.37         0.02           L062         1.13         (a) 50.00         < 0.05	L060	1.10	(c) 0.17	(b) 0.47	- 0.01	-	0.07	0.46
L062         1.13         (a) 50.00         < 0.05	L061	0.90	10.60		0.01	0.04	-0.37	0.02
L069         0.96         39.66         0.12         0.35         -0.23         0.16           L070         1.35         15.40         -         0.01         0.51         0.90           L071         0.90         0.30         0.001         0.10         -0.37         0.02           L073         0.25         (a) 0.15         -         -         -3.15         -2.76           L077         0.87         (c) 0.20         -         -         -0.44         -0.05           L078         1.48         (a) (c) 0.13         <0.01         0.04         -0.63         -0.23           L081         1.43         (a) 0.23         -         -         -0.64         1.03           L082         0.90         0.24         0.01         0.04         -0.63         -0.23           L082         0.90         0.24         0.01         0.04         -0.37         0.02           L083         0.68         (a) 0.20         0.01         0.05         -0.98         -0.58           L085         0.98         0.34         0.01         0.05         -0.18         0.21           L087         0.92         (b) 0.00         -         -	L062	1.13	(a) 50.00		< 0.05	0.10	0.12	0.52
L070       1.35       15.40       -       0.01       0.51       0.90         L071       0.90       0.30       0.001       0.10       -0.37       0.02         L073       0.25       (a) 0.15       -       -       -3.15       -2.76         L077       0.87       (c) 0.20       -       -       -0.44       -0.05         L078       1.48       (a) (c) 0.13       <0.01       0.04       -0.63       -0.23         L081       1.43       (a) 0.23       -       -       0.64       1.03         L082       0.90       0.24       0.01       0.04       -0.63       -0.23         L083       0.68       (a) 0.20       0.01       0.05       -0.98       -0.58         L085       0.98       0.34       0.01       0.05       -0.18       0.21         L087       0.92       (b) 0.00       -       -       -0.32       0.07         L096       1.30       (b) 0.15       0.02       0.20       0.43       0.82         L097       1.98       17.25       0.04       0.10       1.34       1.74	L069	0.96	39.66		0.12	0.35	-0.23	0.16
L071       0.30       0.30       0.001       0.10       0.01       0.01       0.01         L073       0.25       (a) 0.15       -       -       -3.15       -2.76         L077       0.87       (c) 0.20       -       -       -0.44       -0.05         L079       0.80       7.10       0.01       0.04       -0.63       -0.23         L081       1.43       (a) 0.23       -       -       0.64       1.03         L082       0.90       0.24       0.01       0.04       -0.37       0.02         L083       0.68       (a) 0.20       0.01       0.05       -0.98       -0.58         L085       0.98       0.34       0.01       0.05       -0.18       0.21         L087       0.92       (b) 0.00       -       -       -0.32       0.07         L096       1.30       (b) 0.15       0.02       0.20       0.43       0.82         L097       1.98       17.25       0.04       0.10       1.34       1.74	L070	1.35	15.40	0.30	- 0.001	0.01	0.51	0.90
L077         0.87         (c) 0.20         -         -         -0.44         -0.05           L078         1.48         (a) (c) 0.13         < 0.01	L073	0.30		(a) 0.15	0.001	0.10	-3.15	-2.76
L078         1.48         (a) (c) 0.13         < 0.01	L077	0.87	(c) 0.20	(u) 0.10	-	-	-0.44	-0.05
L079         0.80         7.10         0.01         0.04         -0.63         -0.23           L081         1.43         (a) 0.23         -         -         0.64         1.03           L082         0.90         0.24         0.01         0.04         -0.37         0.02           L083         0.68         (a) 0.20         0.01         0.05         -0.98         -0.58           L085         0.98         0.34         0.01         0.05         -0.18         0.21           L087         0.92         (b) 0.00         -         -         -0.32         0.07           L096         1.30         (b) 0.15         0.02         0.20         0.43         0.82           L097         1.98         17.25         0.04         0.10         1.34         1.74	L078	1.48	(a) (c) 0.13		< 0.01	< 0.10	0.71	1.10
L081       1.43       (a) 0.23       -       -       0.64       1.03         L082       0.90       0.24       0.01       0.04       -0.37       0.02         L083       0.68       (a) 0.20       0.01       0.05       -0.98       -0.58         L085       0.98       0.34       0.01       0.05       -0.18       0.21         L087       0.92       (b) 0.00       -       -       -0.32       0.07         L096       1.30       (b) 0.15       0.02       0.20       0.43       0.82         L097       1.98       17.25       0.04       0.10       1.34       1.74	L079	0.80	7.10		0.01	0.04	-0.63	-0.23
L062         0.50         0.24         0.01         0.04         -0.37         0.02           L083         0.68         (a) 0.20         0.01         0.05         -0.98         -0.58           L085         0.98         0.34         0.01         0.05         -0.18         0.21           L087         0.92         (b) 0.00         -         -         -0.32         0.07           L096         1.30         (b) 0.15         0.02         0.20         0.43         0.82           L097         1.98         17.25         0.04         0.10         1.34         1.74	L081	1.43		(a) 0.23	-	-	0.64	1.03
L085         0.98         0.34         0.01         0.05         -0.36         0.21           L087         0.92         (b) 0.00         -         -         -0.32         0.07           L096         1.30         (b) 0.15         0.02         0.20         0.43         0.82           L097         1.98         17.25         0.04         0.10         1.34         1.74	LU02	0.90		0.24 (a) 0.20	0.01	0.04	-0.37 _0 98	0.02 -0.58
L087         0.92         (b) 0.00         -         -         -0.32         0.07           L096         1.30         (b) 0.15         0.02         0.20         0.43         0.82           L097         1.98         17.25         0.04         0.10         1.34         1.74	L085	0.98		0.34	0.01	0.05	-0.18	0.21
L096         1.30         (b) 0.15         0.02         0.20         0.43         0.82           L097         1.98         17.25         0.04         0.10         1.34         1.74           L097         0.51         0.05         0.04         0.10         1.34         1.74	L087	0.92		(b) 0.00	-	-	-0.32	0.07
L097 1.98 17.25 0.04 0.10 1.34 1.74	L096	1.30		(b) 0.15	0.02	0.20	0.43	0.82
	L097	1.98	17.25		0.04	0.10	1.34	1.74
L100 0.01 - 0.05 0.10 -1.20 L101 1.20 0.13 0.26 0.65	L100	1.20		- 0.13	0.05	0.10	-1.60	-1.21

**Table 10**: z-scores for event TC1507 maize powder level 1 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> 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 underestimated. Results are as submitted by participants.

		Ма	ize event	TC1507			
			Robust me	an = <i>0.26 cp</i>	о/ср %		
Laboratory			Assigned v	alue = 0.19 c	:р/ср %		
number	Value	Uncerta	ninty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L005	0.44		0.09	-	-	1.12	1.81
L009	1.21	(e) 1.88		-	-	3.31	4.01
L024	0.12		(a) 0.34	0.01	0.10	-1.71	-1.01
L026	0.40		(a) 0.06	-	-	0.91	1.61
L028	0.14		0.05	0.05	0.10	-1.37	-0.67
L029	0.58	(a) (c) 0.09		-	-	1.72	2.41
L030	0.16	(c) 0.05		0.01	0.10	-1.08	-0.38
L032	0.30	37.00		0.05	0.10	0.28	0.98
L039	0.23	(b) 0.28		0.10	0.10	-0.29	0.40
L043	1.11	(a) 20.50		-	-	3.13	3.82
L046	0.96	14.95		-	-	2.82	3.51
L049	0.08	(c) 0.03		0.001	0.01	-2.59	-1.89
L052	0.32		-	-	-	0.42	1.12
L054	0.37		0.20	0.05	0.10	0.74	1.44
L060	0.20	(c) 0.09		-	-	-0.60	0.10
L063	0.34		0.30	< 1 cp	10 ср	0.56	1.25
L065	0.10	(a) 25.00		-	-	-2.10	-1.41
L066	0.14		(a) 0.02	-	-	-1.37	-0.67
L067	0.35		0.15	0.04	0.06	0.62	1.32
L068	0.36		(a) 0.12	-	-	0.68	1.38
L074	0.15	20.00		-	-	-1.22	-0.52
L076	0.22	25.00		2 cp	0.01	-0.39	0.31
L077	0.28	(c) 0.10		-	-	0.13	0.83
L080	0.30	-		-	-	0.28	0.98
L083	0.17		(a) 0.05	-	-	-0.95	-0.25
L088	0.70	(a) 23.20		0.01	0.05	2.12	2.82
L089	0.12	(a) 25.00		-	-	-1.71	-1.01
L095	0.20	-		0.03	0.05	-0.60	0.10
L098	> 0.01	-		0.01	-	*	*
L099	0.19		(a) 0. <u>11</u>			-0.71	-0.01

**Table 11**: z-scores for event TC1507 maize powder level 2 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> 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. Results are as submitted by participants.

		Mai	ize event	: TC1507					
			Robust me	ean = 0.71 c <sub>l</sub>	o/cp %				
Laboratory	Assigned value = 0.43 cp/cp %								
number	Value	Uncerta	inty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>		
		relative	absolute						
L005	1.14		0.39	-	-	1.02	2.12		
L009	3.86	10.86		-	-	3.67	4.77		
L024	0.34		(a) 0.52	0.01	0.10	-1.61	-0.51		
L026	1.08		(a) 0.11	-	-	0.90	2.00		
L028	0.38		0.05	0.05	0.10	-1.37	-0.26		
L029	1.56	(a) (c) 0.27		-	-	1.70	2.80		
L030	0.49	(c) 0.10		0.01	0.10	-0.81	0.29		
L032	0.80	37.00		0.05	0.10	0.25	1.35		
L039	0.81	(b) 0.28		0.10	0.10	0.28	1.38		
L043	3.31	(a) 20.50		-	-	3.33	4.44		
L046	0.44	24.04		-	-	-1.07	0.03		
L049	0.27	(c) 0.11		0.00	0.01	-2.11	-1.01		
L052	0.83		-	-	-	0.33	1.43		
L054	1.00		0.50	0.05	0.10	0.74	1.84		
L060	0.55	(c) 0.14		-	-	-0.56	0.54		
L063	0.91		0.31	< 1 cp	10 cp	0.53	1.63		
L065	0.58	(a) 25.00		-	-	-0.45	0.65		
L066	0.44		(a) 0.05	-	-	-1.04	0.06		
L067	1.00		0.30	0.04	0.06	0.74	1.84		
L068	0.74		(a) 0.67	-	-	0.08	1.18		
L074	0.43	36.00		-	-	-1.10	0.00		
L076	0.75	25.00		2 cp	0.01	0.11	1.21		
L077	0.97	(c) 0.20		-	-	0.67	1.77		
L080	1.00	-		-	-	0.74	1.84		
L083	0.68		(a) 0.20	-	-	-0.10	1.00		
L088	2.10	(a) 23.20		0.01	0.05	2.35	3.45		
L089	0.59	(a) 25.00		-	-	-0.41	0.69		
L095	0.50	-		0.03	0.05	-0.77	0.33		
L098	> 0.01	-		0.01	-	*	*		
L099	0.54		(a) 0.32	-	-	-0.60	0.50		

**Table 12**: z-scores for event MIR604 maize powder level 1 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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, (e) *U* seems to be underestimated. Results are as submitted by participants.

	Maize event MIR604								
	Robust mean = 3.15 m/m %								
Laboratory	Value		Assigned val	ue = 3.38	m/m %	1	2		
number	value		absolute		LOQ m/m	z-score	z-score		
L002	2.95	Telative	(a) 0.26	-	-	-0.14	-0.30		
L003	2.69		1.21	0.04	0.10	-0.34	-0.50		
L005	4.84		0.83	0.05	0.20	0.93	0.78		
L006	4.90		(b) 2.90	-	-	0.96	0.81		
L007	6.70		(b) 3.90	< 0.10	0.10	1.64	1.49		
L008	2.11	04.75	(a) 0.70	-	-	-0.87	-1.02		
L009	10.70	34.75	(2) 1 15	- 0.01	- 0.10	3.03	3.48 0.26		
	9.47		(a) 1.43	0.01	0.10	2.39	2 24		
L012	2.58		1.02	0.02	0.08	-0.43	-0.59		
L015	2.93	(c) 0.88		0.02	0.05	-0.16	-0.31		
L017	2.57	26.00		0.09	0.09	-0.44	-0.59		
L018	0.87		(a) 0.28	-	-	-2.79	-2.95		
L019	0.76		0.25	-	-	-3.09	-3.24		
L020	3.72		0.76	0.02	0.20	0.36	0.21		
L021	0.72	28.00		-	-	-3.20	-3.36		
L023	3.40		0.95	0.03	0.10	0.17	0.01		
L024	3.4Z 2.70	(a) 0.14	(a) 0.00	0.02	0.10	0.18	0.03		
1 026	2.70	(0) 0.14	(a) 0.00	0.04	0.10	-0.33	-0.49		
L030	2.72	-	(a) 0.00	-	-	-0.32	-0.47		
L031	3.84		(a) 1.13	-	0.10	0.43	0.28		
L033	3.12		0.36	0.05	0.12	-0.02	-0.17		
L034	2.53	(a) 18.00		-	-	-0.48	-0.63		
L035	3.30	(c) 0.26		0.01	0.10	0.10	-0.05		
L036	16.09		2.58	-	-	3.54	3.39		
L037	3.86		1.04	0.10	0.20	0.44	0.29		
L038	3.16		(b) 0.17	0.03	0.10	0.01	-0.15		
	2.82	(c) 0.34	0.28	0.02	0.05	-0.24	-0.39		
1 044	2.00	(b) (c) $0.34$		0.02	0.05	-0.42	-0.37		
L045	4.01	(6) (6) 0.12	0.18	- 0.00	-	0.53	0.37		
L047	2.56	(a) (c) 0.64		0.05	0.10	-0.45	-0.60		
L050	3.49	25.77		0.05	0.10	0.22	0.07		
L051	2.97		(a) 0.56	0.05	0.10	-0.13	-0.28		
L055	19.32	(e) 2.21		0.01	0.05	3.94	3.79		
L056	2.98		0.78	< 0.10	0.10	-0.12	-0.27		
L058	2.80		(b) 1.07	0.01	0.05	-0.26	-0.41		
L059	3.11	(a) 0.05	(D) 1.19	0.01	0.05	-0.03	-0.18		
1 061	3.00	(0) 0.05		0.01	0.04	-0.02	-0.17		
L062	2.77	(a) 32.00		- 0.01	- 0.04	-0.28	-0.20		
L063	2.99	(4) 02100	1.11	< 0.05	< 0.09	-0.11	-0.27		
L069	3.36	30.24		0.07	0.23	0.14	-0.01		
L070	3.80	13.60		-	0.01	0.41	0.25		
L071	2.50		0.80	0.001	0.02	-0.50	-0.65		
L077	4.08	(c) 1.00		-	-	0.56	0.41		
L078	1.88	(a) (c) 0.15		< 0.05	< 0.08	-1.12	-1.27		
L079	2.70	7.20		0.01	0.04	-0.33	-0.49		
LU81	3.11		(a) 0.00	-	-	-0.03	-0.18		
LU02	3.31 3.00		U.18 (a) 1.17	0.03	0.09	0.11	-U.U5 0 21		
1 085	2.79		(a) 1.17 0.30	0.01	0.05	-0.40	-0.31		
L087	3.91		(b) 0.00		-	0.47	0.32		
L096	2.60		(b) 0.30	0.02	0.20	-0.42	-0.57		
L097	4.29	28.51	. /	0.04	0.10	0.67	0.52		
L100	3.47		-	0.05	0.10	0.21	0.06		
L101	3.10		0.20	-	-	-0.03	-0.19		

**Table 13**: z-scores for event MIR604 maize powder level 2 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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) seems to be a typing mistake. Results are as submitted by participants.

	Maize event MIR604								
	Robust mean = 0.91 m/m %								
Laboratory			Assign	ned va	lue = 0.89	m/m %			
number	Value	Uncerta	inty		LOD m/m	LOQ m/m	z-score <sup>1</sup>	z-score <sup>2</sup>	
		relative	abs	olute					
L002	1.22		(a)	0.08	-	-	0.63	0.68	
L003	0.78			0.37	0.04	0.10	-0.34	-0.29	
L005	1.38			0.66	0.05	0.20	0.90	0.95	
L006	0.78		(b)	0.09	-	-	-0.34	-0.29	
L007	1.08		(b)	0.13	< 0.10	0.10	0.37	0.42	
L008	0.63		(a)	0.26	-	-	-0.80	-0.75	
L009	5.04	21.76			-	-	3.71	3.77	
L011	1.44		(a)	0.55	0.01	0.10	0.99	1.04	
L012	3.06		(a)	0.00	0.10	0.10	2.63	2.68	
L013	0.83			0.33	0.02	0.08	-0.20	-0.15	
L015	0.79	(c) 0.24			0.02	0.05	-0.31	-0.26	
L017	0.71	26.00			0.09	0.09	-0.54	-0.49	
L018	0.26		(a)	0.08	-	-	-2.72	-2.67	
L019	0.16			0.05	-	-	-3.78	-3.73	
L020	0.90	10.00		0.19	0.02	0.19	-0.03	0.02	
L021	2.59	19.00		0.00	-	-	2.27	2.32	
L023	0.93		(-)	0.26	0.03	0.10	0.04	0.10	
L024	0.98	(a) 0.16	(a)	0.00	0.02	0.10	0.16	0.21	
L025	0.00	(0) 0.10	$(\mathbf{a})$	0.00	0.04	0.10	-0.91	-0.00	
	0.00	_	(a)	0.00	-	-	-0.91	-0.00	
1 031	0.00	_	(2)	0 27		0.10	-0.00	-0.02	
1 033	0.50		(a)	0.21	0.05	0.10	-0.03	0.02	
1 034	0.34	(a) (f) 247 00		0.24	0.00	0.12	-0.28	-0.23	
1 035	1 40	(a) (1) 2 + 1.00 (c) 0.08			0.01	0.10	0.93	0.98	
L036	5.98	(0) 0.00		1 98	-	-	4.08	4.14	
L037	1.23			0.08	0.10	0.20	0.65	0.70	
L038	0.92		(b)	0.04	0.03	0.10	0.02	0.07	
L040	0.83		()	0.21	0.02	0.05	-0.20	-0.15	
L041	0.75	(c) 0.13			0.02	0.05	-0.42	-0.37	
L044	0.76	(b) (c) 0.07			0.05	0.09	-0.39	-0.34	
L045	1.25			0.12	-	-	0.69	0.74	
L047	0.35	(a) (c) 0.11			0.05	0.10	-2.08	-2.03	
L050	0.89	28.72			0.05	0.10	-0.06	-0.01	
L051	0.96		(a)	0.21	0.05	0.10	0.11	0.16	
L055	5.59	(c) 0.73			0.01	0.05	3.94	3.99	
L056	0.75			0.30	< 0.10	0.10	-0.42	-0.37	
L058	0.92		(b)	0.35	0.01	0.05	0.02	0.07	
L059	0.92		(b)	0.35	0.01	0.04	0.02	0.07	
L060	0.90	(c) 0.16			-	-	-0.03	0.02	
L061	0.90	10.20			0.01	0.04	-0.03	0.02	
L062	0.78	(a) 32.00			-	-	-0.34	-0.29	
L063	0.97			0.68	< 0.05	< 0.09	0.13	0.19	
L069	1.03	67.80			0.07	0.23	0.27	0.32	
L070	1.10	16.30			-	0.01	0.41	0.46	
L071	0.70	(-) 0.00		0.20	0.001	0.03	-0.57	-0.52	
L077	1.19	(c) 0.30			-	-	0.58	0.63	
L078	0.49	(a) (c) 0.15			< 0.05	< 0.08	-1.35	-1.30	
	0.90	6.50	(-)	0.04	0.01	0.04	-0.03	0.02	
	0.72		(a)	0.01	-	-	-0.51	-0.46	
	0.92		$\langle \alpha \rangle$	0.09	0.03	0.09	0.02	0.07	
LU03	1.07		(a)	0.32	0.01	0.05	0.30	0.40	
	1.00		(h)	0.20	0.01	0.05	-0.13	-0.07	
1 096	0.70		(D) (b)	0.00	- 0.02	- 0.20	0.24 -0 57	0.30 _0.50	
1 097	0.70	3/ 00	(u)	0.10	0.02	0.20	0.57	-0.5Z 0.1/	
1 100	0.35	54.99		-	0.04	0.10	-0.09	_0.14	
L101	0.98			0.10	0.00		0.16	0.21	
				0.10				· · · · ·	
**Table 14**: z-scores for event MIR604 maize powder level 1 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> z-score calculated on the basis of the assigned value, \* = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (c) *U* seems to be an absolute value. Results are as submitted by participants.

		Ма	nize even	t MIR604			
			Robust me	ean = 2.57 c <b>i</b>	о∕ср %		
Laboratory			Assigned v	alue = 1.34 c	p/cp %		
number	Value	Uncerta	ainty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L005	4.84		0.83	-	-	1.37	2.79
L009	8.38	34.75		-	-	2.56	3.98
L024	1.71		(a) 0.00	0.02	0.10	-0.89	0.53
L026	2.02		(a) 0.57	-	-	-0.53	0.89
L028	0.80		0.15	0.05	0.10	-2.54	-1.12
L030	1.36	(c) 0.20		0.01	0.10	-1.39	0.03
L032	1.30	31.00		0.05	0.10	-1.48	-0.06
L043	5.63	(a) 22.30		-	-	1.70	3.12
L046	1.20	16.00		-	-	-1.66	-0.24
L052	1.62		-	-	-	-1.01	0.41
L054	3.77		2.00	0.05	0.10	0.83	2.25
L060	1.56	(c) 0.18		-	-	-1.09	0.33
L065	9.07	(a) 25.00		-	-	2.73	4.15
L066	0.99		(a) 0.11	-	-	-2.08	-0.66
L067	3.00		0.50	0.04	0.06	0.33	1.75
L068	3.64		(a) 1.77	-	-	0.75	2.17
L074	1.80	14.00		-	-	-0.78	0.64
L076	3.22	25.00		33 cp	0.09	0.49	1.91
L080	3.40	-		-	-	0.60	2.02
L083	3.90		(a) 1.17	-	-	0.90	2.32
L088	3.69	(a) 15.50		0.01	0.05	0.78	2.20
L089	9.23	(a) 25.00		-	-	2.77	4.19
L095	0.97	-		0.03	0.05	-2.12	-0.70
L098	> 0.01	-		0.01	-	*	*

**Table 15**: z-scores for event MIR604 maize powder level 2 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, <sup>1</sup> z-score calculated on the basis of the robust mean is reported for information purpose only, <sup>2</sup> z-score calculated on the basis of the assigned value, \* = no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (c) *U* seems to be an absolute value. Results are as submitted by participants.

		Ма	ize event	t MIR604			
			Robust me	ean = <i>0.85 c</i>	о∕ср %		
Laboratory			Assigned v	alue = 0.34 d	p/cp %		
number	Value	Uncerta	inty	LOD cp/cp	LOQ cp/cp	z-score <sup>1</sup>	z-score <sup>2</sup>
		relative	absolute				
L005	1.38		0.66	-	-	1.04	3.02
L009	2.52	21.76		-	-	2.35	4.33
L024	0.49		(a) 0.00	0.02	0.10	-1.21	0.77
L026	0.60		(a) 0.16	-	-	-0.77	1.21
L028	0.24		0.07	0.05	0.10	-2.75	-0.78
L030	0.44	(c) 0.09		0.01	0.10	-1.44	0.54
L032	0.40	31.00		0.05	0.10	-1.65	0.33
L043	2.18	(a) 22.30		-	-	2.04	4.01
L046	2.71	6.53		-	-	2.51	4.48
L052	0.33		-	-	-	-2.06	-0.09
L054	1.02		0.50	0.05	0.10	0.39	2.36
L060	0.45	(c) 0.13		-	-	-1.39	0.59
L065	3.07	(a) 25.00		-	-	2.78	4.76
L066	0.34		(a) 0.04	-	-	-2.02	-0.04
L067	1.00		0.30	0.04	0.06	0.34	2.32
L068	0.88		(a) 0.27	-	-	0.07	2.04
L074	0.52	53.00		-	-	-1.08	0.90
L076	0.95	25.00		33 cp	0.09	0.23	2.21
L080	1.10	-		-	-	0.55	2.53
L083	1.07		(a) 0.32	-	-	0.49	2.47
L088	1.33	(a) 15.50		0.01	0.05	0.96	2.94
L089	3.39	(a) 25.00		-	-	2.99	4.97
L095	0.30	-		0.03	0.05	-2.27	-0.29
L098	> 0.01	-		0.01	-	*	*



Figure 6: z-scores for maize event GA21 powder level 1 on the basis of an assigned value of 0.26 m/m % (□) and a robust mean of 0.26 m/m % (◊).

EURL-CT-02/11final CTRb



Figure 7: z-scores for maize event GA21 powder level 2 on the basis of an assigned value of 2.08 m/m % (□) and a robust mean of 1.92 m/m % (◊).



**Figure 8**: z-scores for maize event GA21 powder level 1 on the basis of an assigned value of 0.14 cp/cp % ( $\Box$ ) and a robust mean of 0.31 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.



**Figure 9**: z-scores for maize event GA21 powder level 2 on the basis of an assigned value of 0.86 cp/cp % ( $\Box$ ) and a robust mean of 1.76 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.



Figure 10: z-scores for maize event TC1507 powder level 1 on the basis of an assigned value of 0.30 m/m % (□) and a robust mean of 0.38 m/m % (◊).



Figure 11: z-scores for maize event TC1507 powder level 2 on the basis of an assigned value of 0.89 m/m % (□) and a robust mean of 1.07 m/m % (◊).



**Figure 12**: z-scores for maize event TC1507 powder level 1 on the basis of an assigned value of 0.19 cp/cp % ( $\Box$ ) and a robust mean of 0.26 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.



**Figure 13**: z-scores for maize event TC1507 powder level 2 on the basis of an assigned value of 0.43 cp/cp % ( $\Box$ ) and a robust mean of 0.71 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.



Figure 14: z-scores for maize event MIR604 powder level 1 on the basis of an assigned value of 3.38 m/m % (□) and a robust mean of 3.15 m/m % (◊).



Figure 15: z-scores for maize event MIR604 powder level 2 on the basis of an assigned value of 0.89 m/m % (□) and a robust mean of 0.91 m/m % (◊).



**Figure 16:** z-scores for maize event MIR604 powder level 1 on the basis of an assigned value of 1.34 cp/cp % ( $\Box$ ) and a robust mean of 2.57 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.

#### EURL-CT-02/11final CTRb



**Figure 17**: z-scores for maize event MIR604 powder level 2 on the basis of an assigned value of 0.34 cp/cp % ( $\Box$ ) and a robust mean of 0.85 cp/cp % ( $\diamond$ ). The z-scores calculated on the basis of the robust mean are shown for information purpose only.

## 7. 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  $\sigma$  the more likely participants with a z-score outside the working range of -2 to +2 are underperforming. However, a greater  $\sigma$  will also increase the probability of accepting unsatisfactory measurement results. Hence a compromise should be made between the choice of the value of  $\sigma$  and the attempt to assess the participants' performance. In any case a z-score outside the working range of -3 to +3 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.

# 8. Evaluation of results

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in m/m % for both maize powder levels 1 and 2 regardless of the GM event. However, a lower percentage (43 -86 %) 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 very close to the robust means expressed in m/m % (Figure 18a). There was however a disparity between the assigned values obtained through digital PCR and the robust means expressed in cp/cp % (Figure 18b). The majority of these participants 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 mass fraction percentage when a Certified Reference Material (CRM), certified for the mass fraction is used as calibrant. Indeed, all available CRMs (i.e. ERM-BF414, ERM-BF418 and ERM-BF423) have been certified for the GM mass fraction and not for the DNA copy number ratio<sup>(19, 20, 21)</sup>. 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<sup>(19, 20, 21)</sup> 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  $4^{(22)}$ . As a consequence the robust means ( $\mu_R$ ) and assigned values ( $\mu$ ) 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 6, 7, 10, 11, 14 and 15).





**Figure 18**: Comparison of assigned values ( $\mu$ ) and robust means ( $\mu_R$ ) of the maize powder levels 1 and 2 test items in m/m % (a) and in cp/cp % (b). m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, L1 = level 1, L2 = level 2. The error bars represent the expanded uncertainties.

An overview of the laboratories having obtained outlying z-scores is provided in Table 16.

Table 16: Overview of laboratories with outlying z-scores on the basis of the assigned value for the maize powder levels 1 and 2 test items in m/m % (a) and in cp/cp % (b). - = no results reported.

2	١
a	)
	-

		Outl	ying z-s	cores [r	n/m %]	
Laboratory	G	A21	ТС	:1507	MI	R604
number	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
L007			Х			
L008						
L009	х	х	х	х	х	х
L012					х	х
L018					х	х
L019					х	х
L021					х	х
L036		х			х	х
L042				х	-	-
L047						х
L050		х				
L055			х	х	Х	х
L073	х	х	х	х	-	-

		Outly	ving z-so	cores [c	p/cp %]	
Laboratory	G	A21	тс	:1507	MI	R604
number	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
L005	х	х		х	Х	х
L009	х	х	х	х	х	х
L010	х	х	-	-	-	-
L026	х	х		х		
L029			х	х	-	-
L043			х	х	х	х
L046	х		х			х
L052	х	х				
L054					х	х
L065	х	х			х	х
L067	х	х				х
L068	х	х			х	х
L076						х
L080					х	х
L083					Х	х
L088			Х	х	х	х
L089	х	х			х	х

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 17.

Laboratori	olem with calibration curve	alues outside working range	utside range	e outside range	at DNA amount analysed	pped results	y/paste error	sible reporting error	sible calculation mistake	itical values for m/m % and p %	itive NTC
number	lo	2 7	2 <sup>2</sup> 0		ê.C	Swa	do	soc	soc	der p/c	soc
L005		0	<u>u</u>	0)	0	0)	<u> </u>			<u> </u>	<u> </u>
L007								х			
L008	х		х								
L009	х		х	х							
L010					х						
L012	х			Х							
L018					х						
L019					Х						
L021						х					
L026										х	
L029				Х							
L036		х		Х							х
L042				Х							
L043				Х	Х						
L046		х				х					
	v	X					X				
L050 L052	x	X			v				v		
1 054	^				^			Y	^		
1 055	x	x	x	x				~			
L065 + L089	x	x	x	x	x						
L067	~		~					х			
L068				х							х
L073			х	х							
L076					х						
L080								х			
L083				х						х	
L088								Х			

**Table 17**: Overview of the possible reasons for outlying z-scores. Ct value = cycle threshold value,  $R^2$  = coefficient of determination, NTC = no template control.

In this section the terms used in Table 17 are further explained.

- 'Problem with calibration curve' refers to the standards of the dilution series, in that the measured Ct diverged from the extrapolated Ct value<sup>(23)</sup>.
- '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<sup>2</sup> outside range' implies that the coefficient of determination (R<sup>2</sup>) was poor compared to the acceptable value (R<sup>2</sup>  $\ge$  0.98) as outlined in the ENGL guidance document<sup>(23)</sup>.
- 'Slope outside range' indicates that the slope of the calibration curve was poor compared to the acceptable values (-3.6  $\leq$  slope  $\leq$  -3.1) as outlined in the ENGL guidance<sup>(23)</sup>.
- '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' means that the participant has swapped the results reported for the maize powder levels 1 and 2 test items.
- `Copy/paste error' refers to a reporting mistake made during the on-line submission of results.
- 'Possible reporting error' may either indicate that those participants should have reported their results in m/m % instead of cp/cp % or that IRMM's guidelines<sup>(22)</sup> for the conversion of m/m % to cp/cp % were not taken into account.
- 'Possible calculation mistake' refers to the observation that a re-calculation of the sample intake for real-time PCR on the basis of the information provided by the participant did not produce the same result.
- 'Identical values for m/m % and cp/cp %' indicates that those participants reported identical values for the GM content expressed in m/m % and in cp/cp %.
- 'Positive NTC' (i.e. no template control) means that amplification was noted for the negative control.

# 9. **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 9.1 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 9.2).

### 9.1 NRLs

One NRL (L045) reported a false negative result for the GA21 event maize powder level 2 test item and one NRL (L056) reported false positive results. As L045 quantified the GA21 content of the level 2 test item, it is suspected that it concerns a reporting mistake. Out of 62 NRLs

two (L064 and L086) only performed screening analyses whereas three NRLs (L010, L029 and L084) did not quantify all three GM events. This implies that 92 % of NRLs quantified all three GM events.

L010 only screened for events Bt11, GA21, MON 810, MON 863 and NK603. They only quantified event GA21 using the EU-RL GMFF validated event-specific real-time PCR quantification method<sup>(24)</sup>. L064 reported to have only qualitative PCR methods for events MON 810, NK603, Bt11 and Bt176.

Seventy NRLs were invited to participate in this comparative testing round. One NRL was not invited because its Ministry of Agriculture announced the cancellation of its NRL in the field of GMOs. Seven NRLs (of which one is no longer a NRL) declined participation. One (L104) out of 63 NRLs that registered for the fourth comparative testing round did not report results. Due to a delay in the delivery of reference materials and reagents the NRL could not submit results within the deadline.

Eighteen (L005, L007, L009, L010, L018, L019, L021, L026, L029, L043, L046, L047, L050, L067, L068, L076, L080 and L088) out of 62 NRLs, obtained z-scores outside the working range of -2 to +2. Eleven (L005, L010, L026, L029, L043, L046, L067, L068, L076, L080 and L088) of those laboratories had expressed the results in cp/cp %. Six laboratories (L007, L018, L019, L021, L047 and L050) had expressed the results in m/m % and one participant (L009) had obtained outlying z-scores for both measurement units. The z-scores of seven NRLs (L007, L009, L018, L019, L021, L047 and L050) that had expressed the results in m/m % were outside the range of -2 to +2 when calculated on the basis of the assigned value derived from the homogeneity study (Tables 4, 5, 8, 9, 12 and 13). Analysing the raw data of these participants allowed the identification of possible causes for these results. The z-scores of seven NRLs (L005, L010, L026, L029, L067, L076 and L080) only became unsatisfactory after it had been decided that the z-scores should be calculated on the basis of the assigned value obtained by digital PCR. If time had permitted, these laboratories would have been asked to repeat the experimental work.

L009 took account of the zygosity statement to convert the results expressed in m/m % to cp/cp %. However, there was a systematic overestimation of the GM content and the copy numbers of the calibration curves were different for experiments carried out on different days. In addition, the MIR604 calibration curve did not comply with the recommendations for the R<sup>2</sup> coefficient and the slope outlined in the ENGL guidance document<sup>(23)</sup>. L088 reported results in m/m % for GM event GA21 and in cp/cp % for GM events TC1507 and MIR604. L088 confirmed their intention to submit results for different GM events in different units. It is however recommended that participants express their measurements results in m/m % when a Certified Reference Material (CRM), certified for the mass fraction is used as calibrant. They should take account of the zygosity stated in the certification report<sup>(19, 20, 21)</sup> and should closely follow IRMM's guidelines for the conversion of mass fraction to DNA copy number ratio<sup>(22)</sup>. L005 and L026 should closely follow IRMM's guidelines<sup>(22)</sup> when converting their results from m/m % into cp/cp % because they reported identical values for both measurement units. The same holds true for L029 because this participant reported almost identical values for the GM content expressed in m/m % and cp/cp %. L010 only quantified

GM event GA21 which was overestimated by a factor of 4. When asked to submit their raw data, L047 reported a copy/paste error in the quantification value of event MIR604 for the level 2 test item. The analysis of their raw data by the EU-RL GMFF confirmed this error. The corrected value was subsequently inserted in Table 20a. It is suspected that L067, L076 and L080 expressed the results in m/m % rather than in cp/cp % because of an overestimation of the GM content by a factor of 2 or more. L067 used an event-specific national reference method whereas L076 used the EU-RL GMFF validated event-specific real-time PCR quantification method<sup>(24)</sup>. Both L067 and L080 applied hmg as an endogenous target sequence. L080 confirmed that they had not taken the heterozygosity of maize into account when converting m/m % into cp/cp %. L067 also shows an overestimation by a factor of 4 regarding the quantification of GM event GA21. Moreover, L076 sometimes used sample intakes above 400 ng for real-time PCR and reported results in m/m % for GM event GA21 and in cp/cp % for GM events TC1507 and MIR604. As outlined above sample intakes above 200 ng might give rise to a decrease of PCR efficiency. It is suggested that L067, L076 and L080 pay close attention to the measurement units. The results of L006 and L007 were compared because it concerned the same laboratory. L006 had used the standard curve method whereas L007 had used a delta Ct method. In the case of L006 the GM content of maize event 1507 was overestimated whereas the GM content of the quality control material was underestimated. A correction of the negative bias observed for L006 would lead to an even greater overestimation of the GM content. Since the quality control material was not included in the experimental setup by L007, no conclusions can be drawn regarding the bias. L007 is recommended to check that L006 and L007 have similar PCR efficiencies for both quantification methods (i.e. standard curve method versus delta Ct method) used.

Ten NRLs (L008, L009, L018, L019, L021, L043, L046, L050, L068, and L088) were asked to repeat the experimental work related to this fourth 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 17 for each participant. L021 discovered the reason (namely swapped results) for the outlying z-score by itself. L088 repeated the experimental work before receiving feedback from the EU-RL GMFF. They changed the endogenous target sequence in the experimental setup.

#### 9.2 Non-NRLs

Two non-NRLs (L092, L093) reported false negative results. Four non-NRLs (L013, L022, L091 and L092) reported false positive results.

Ten (L012, L036, L042, L052, L054, L055, L065, L073, L083 and L089) out of 31 non-NRLs, obtained z-scores outside the working range of -2 to +2. Five (L052, L054, L065, L083 and L089) of those laboratories had expressed the results in cp/cp %. Five laboratories (L012, L036, L042, L055 and L073) had expressed the results in m/m %. Analysing the raw data of those participants allowed identifying possible causes for these results. Since it was decided only at a later stage in the study to calculate the z-scores on the basis of the assigned value obtained by digital PCR, three non-NRLs (L042, L054 and L083) were not asked to repeat the experimental work. In the case of L042 the slope of the event TC1507 calibration curve was

poor compared to the acceptable values (-3.6  $\leq$  slope  $\leq$  -3.1) as outlined in the ENGL guidance<sup>(23)</sup>. In addition, L042 did not report any values for the slopes and R<sup>2</sup> coefficients of the endogenous targets. It is suggested that L083 follows IRMM's guidelines<sup>(22)</sup> when converting their results from m/m % into cp/cp % because they reported identical values for both measurement units. L052 is suggested to look at its calculations because of the observation that a re-calculation of the sample intake for real-time PCR on the basis of the information provided by the participant did not produce the same result. L052 used the EU-RL GMFF validated event-specific real-time PCR quantification method<sup>(24)</sup>. L054 is suspected to have expressed the results in m/m % rather than in cp/cp % because of a systematic overestimation of the GM content by a factor of 2. L054 used an event-specific real-time PCR quantification method published in a peer reviewed journal, applying *hmg* as the endogenous target sequence. This participant should pay close attention to the measurement units.

Seven non-NRLs (L012, L036, L052, L055, L065, L073, and L089) were asked to repeat the experimental work related to this fourth 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 17 for each participant.

## 9.3 Results of the repetition of the experimental work

The results of the repetition of the experimental work are depicted in Tables 18 to 20. 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 18**: Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for maize event GA21 powder levels 1 and 2. <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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.

	Μ	aize event GA	21	
		Robust mean = 0	).24 m/m %	1
Laboratory		Assigned value =	0.26 m/m %	6
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L008	0.29	0.12	0.30	0.19
L012	0.22	0.00	-0.18	-0.29
L036	0.17	20.67	-0.63	-0.74
L050	0.26	58.84	0.09	-0.02
		Robust mean = 1	.82 m/m %	,
		Assigned value =	2.08 m/m %	6
	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L008	2.12	0.81	0.27	0.03
L012	1.62	0.00	-0.20	-0.43
L036	1.30	24.99	-0.58	-0.82
L050	2.11	5.14	0.26	0.02

b)

a)

	Μ	aize event GA	21	
		Robust mean = 0.	.24 ср/ср %	6
Laboratory		Assigned value =	0.14 cp/cp	%
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	0.58	0.14	1.51	2.42
L046	0.22	49.22	-0.20	0.71
L052	0.15	0.00	-0.84	0.07
L065	0.59	-	1.54	2.45
L089	0.64	-	1.68	2.59
		Robust mean = 1.	.75 cp/cp %	0
		Assigned value =	0.86 cp/cp	%
	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	5.46	0.69	1.98	3.22
L046	1.59	19.01	-0.16	1.07
L052	1.04	0.00	-0.90	0.34
L065	7.19	-	2.46	3.70
L089	5.50	-	1.99	3.23

**Table 19**: Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for maize event TC1507 powder levels 1 and 2. <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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.

	Ма	ize event TC1	507	
		Robust mean =	0.38 m/m %	6
Laboratory		Assigned value =	= 0.30 m/m	%
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L050	0.28	30.14	-0.67	-0.17
L055	0.54	0.24	0.78	1.28
		Robust mean =	1.07 m/m %	6
		Assigned value =	= 0.89 m/m	%
	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L050	0.88	22.85	-0.42	-0.02
L055	1.44	0.64	0.65	1.04

b)

	Ма	ize event TC1	507	
		Robust mean = 0	).25 ср/ср 🤅	%
Laboratory		Assigned value =	0.19 cp/cp	%
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	1.19	0.46	3.38	3.97
L043	0.50	20.50	1.49	2.09
L046	0.45	16.82	1.26	1.86
L088	0.49	23.20	1.45	2.05
		Robust mean = 0	).75 ср/ср 🤅	%
Laboratory		Assigned value =	0.43 cp/cp	%
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	3.92	0.36	3.58	4.80
L043	1.58	20.50	1.61	2.83
L046	1.17	16.67	0.95	2.17
L088	1.13	23.20	0.88	2.10

**Table 20**: Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for maize event MIR604 powder levels 1 and 2. <sup>1</sup> z-score calculated on the basis of the robust mean, <sup>2</sup> 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.

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d	)

	Mai	ze event MIF	R604	
		Robust mean =	3.07 m/m %	6
Laboratory		Assigned value :	= 3.38 m/m	%
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L012	3.36	0.00	0.19	-0.01
L018	1.33	0.43	-1.82	-2.03
L019	1.42	0.46	-1.68	-1.88
L021	2.69	42.00	-0.29	-0.50
L036	3.02	6.20	-0.04	-0.24
L055	3.04	1.21	-0.02	-0.23
		Robust mean =	0.87 m/m %	6
		Robust mean = Assigned value :	0.87 m/m % = 0.89 m/m	% %
_	Value	Robust mean = Assigned value = Uncertainty	0.87 m/m % = 0.89 m/m z-score <sup>1</sup>	% % z-score <sup>2</sup>
L012	Value 0.78	Robust mean = Assigned value = Uncertainty 0.00	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25	% <u>z-score<sup>2</sup></u> -0.29
L012 L018	Value 0.78 0.36	Robust mean = Assigned value = Uncertainty 0.00 0.12	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25 -1.93	% <u>z-score<sup>2</sup></u> -0.29 -1.97
L012 L018 L019	Value 0.78 0.36 0.42	Robust mean = Assigned value = Uncertainty 0.00 0.12 0.14	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25 -1.93 -1.59	% <u>z-score<sup>2</sup></u> -0.29 -1.97 -1.63
L012 L018 L019 L021	Value 0.78 0.36 0.42 0.78	Robust mean =   Assigned value =   Uncertainty   0.00   0.12   0.14   31.00	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25 -1.93 -1.59 -0.25	% <u>z-score<sup>2</sup></u> -0.29 -1.97 -1.63 -0.29
L012 L018 L019 L021 L036	Value 0.78 0.36 0.42 0.78 0.73	Robust mean =   Assigned value =   Uncertainty   0.00   0.12   0.14   31.00   16.40	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25 -1.93 -1.59 -0.25 -0.39	% <u>z-score<sup>2</sup></u> -0.29 -1.97 -1.63 -0.29 -0.43
L012 L018 L019 L021 L036 L047	Value 0.78 0.36 0.42 0.78 0.73 0.74	Robust mean =   Assigned value =   Uncertainty   0.00   0.12   0.14   31.00   16.40   0.11	0.87 m/m 9 = 0.89 m/m z-score <sup>1</sup> -0.25 -1.93 -1.59 -0.25 -0.39 -0.36	% <u>z-score<sup>2</sup></u> -0.29 -1.97 -1.63 -0.29 -0.43 -0.40

b)

Maize event MIR604				
	Robust mean = 2.44 cp/cp %			
Laboratory		Assigned value = 1.34 cp/cp %		
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	8.76	2.09	2.77	4.08
L043	4.77	22.30	1.45	2.76
L046	2.77	5.97	0.28	1.58
L065	3.52	-	0.79	2.10
L089	3.56	-	0.82	2.12
	Robust mean = 0.72 cp/cp %			
Laboratory	Assigned value = 0.34 cp/cp %			
number	Value	Uncertainty	z-score <sup>1</sup>	z-score <sup>2</sup>
L009	3.04	0.76	3.14	4.73
L043	1.32	22.30	1.33	2.92
L046	0.61	16.83	-0.33	1.26
L065	1.76	-	1.96	3.55
L089	0.82	-	0.30	1.89

## 9.3.1 NRLs

With the exception of L018 all NRLs (L008, L019, L021, L047 and L050) that had expressed the results in m/m % obtained satisfactory z-scores upon repetition of the experimental work (Tables 18a-20a).

The NRLs that had expressed the results in cp/cp % and repeated the experimental work did not always improve their performance (Tables 18b-20b). L046 gained z-scores within the range of -2 to +2 for the events GA21 and MIR604. However, the GM content of event TC1507 was overestimated by more than a factor of 2. Likewise L043 and L088 overestimated the GM content of the re-tested events by a factor of at least 2. L068 repeated the experimental work for the quantification of event GA21 but they did not submit results because they claimed to have problems with the EU-RL GMFF validated event-specific quantification method<sup>(24)</sup>.

L009 reported the results of the repetition of the experimental work in cp/cp % only. The overestimation of the GM content of the tested events was in the range of a factor of 4 to 9.

#### 9.3.2 Non-NRLs

With the exception of L073, the non-NRLs (L012, L036 and L055) that had expressed the results in m/m % obtained satisfactory z-scores upon repetition of the experimental work (Tables 18a-20a). The z-scores of the repetition of the experimental work of L073 are not reported because several problems (slopes outside the criteria outlined in the ENGL guidance document<sup>(23)</sup>, Ct values outside the linear working range of the calibration curve, use of a single target plasmid for calibration and low sample intake for real-time PCR of unknown samples) were discovered during the analysis of the raw data. It was suggested that L073 should use a dual-target plasmid for calibration, change the working range of the calibration curves, pay attention to the slopes of the calibration curves and increase the sample intake for real-time PCR of the unknown samples. The second repetition of the experimental work resulted in an improved performance regarding the quantification of the event GA21 (z-scores of 0.39 and 0.73 for the levels 1 and 2 test items, respectively). However, the quantification of the TC1507 event still showed an overestimation of the GM content (z-scores of 3.36 and 1.68 for the levels 1 and 2 test items, respectively).

L052 drastically improved the performance upon repetition of the experimental work. Its zscores for GM event GA21 decreased from > 3 to 0.07 and 0.34 for the levels 1 and 2 test items (Tables 6, 7 and 18b). A single laboratory, represented by L065 and L089, overestimated the GM content of the events GA21 and MIR604 by more than a factor of 2 for both result submissions.

## 10. Conclusions

In this fourth comparative testing round participants were faced with a challenge because they were asked to screen two maize powder test items for ten maize GM events and to determine the GM content of those GM events that were detected. Both test items were produced by the EU-RL GMFF.

Nine percent of participants (L064, L075, L086, L091, L092, L093, L094 and L098) only performed qualitative analyses. At least 91 % of partipants detected maize events GA21 and

EURL-CT-02/11final CTRb

TC1507, whereas about 80 % of participants detected event MIR604 (Figure 5). About 18 % of participants did not screen for event MIR604. At most 3 % of participants did not detect the above-mentioned GM events. With respect to the adventitious presence of GM events about 77 % of participants detected event NK603, whereas 1 % and at most 5 % detected events 59122 and MON 810, respectively. The majority (about 65 % and 56 % for the results expressed in m/m % and cp/cp %, respectively) of participants reported results for the event NK603 in a semi-quantitative way (i.e. < value x). Of those participants that quantified the GM content of event NK603 most participants (i.e. about 82 % and 75 % for the results expressed in m/m % and cp/cp %, respectively) reported a value below 0.1 %. With respect to the 1 % and at most 5 % of participants that detected events 59122 and MON 810, only one and two participants, respectively reported quantitative results for these GM events. Events 3272 and Bt176 were detected by none of the participants whereas at most 5 % and 1 % detected events MON 863 and Bt11, respectively. It can thus be concluded that a majority of participants performed a correct screening with a minority of participants reporting either false positives or negatives (Figure 5).

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 = 4), 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<sup>(25)</sup>.

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in m/m % for both maize powder levels 1 and 2 regardless of the GM event. The assigned values derived from the homogeneity study conducted at the EU-RL GMFF were very close to the robust means expressed in m/m % (Figure 18a). However, a lower percentage (43 - 86 %) 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 obtained through digital PCR and the robust means expressed in cp/cp % (Figure 18b). There was an obvious overestimation of the robust means expressed in cp/cp % (Tables 6, 7, 10, 11, 14 and 15). Unacceptable high z-scores (i.e. z-scores above 2) calculated on the basis of the assigned value were observed in 42 % and 35 % of reported results for GM event GA21, 21 % and 17 % of reported results for GM event TC1507, and 43 % and 57 % of reported results for GM event MIR604 maize powder levels 1 and 2 test items, respectively. With the exception of L005 and L063 all laboratories that expressed their results in cp/cp % prepared a dilution series based on DNA copy numbers. L005 prepared a dilution series in m/m % and L063 used a delta Ct method for real-time PCR quantification. A total of 17 participants that had expressed the results in cp/cp % gained a z-score outside the range of -2 to +2. The majority of these participants 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. Indeed, all available CRMs (i.e. ERM-BF414, ERM-BF418 and ERM-BF423) have been certified for the GM mass fraction and not for the GM copy number ratio<sup>(19, 20, 21)</sup>. 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<sup>(19, 20, 21)</sup> 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  $4^{(22)}$ . The Application Note 4 also explains how to calculate the expanded uncertainty in such cases.

In this comparative testing round, the robust means ( $\mu_R$ ) and assigned values ( $\mu$ ) expressed in cp/cp % are quite different from each other. The z-scores calculated on the basis of the robust means in cp/cp % are given for information purpose only.

The assigned values ( $\mu$ ) in cp/cp %, obtained by digital PCR, were compared with those expressed in m/m % (Tables 4 – 15). As described in the IRMM application note<sup>(22)</sup> 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). The ratio of the assigned values in cp/cp % to those in m/m % were 54 % and 41 % for GM event GA21, 63 % and 48 % for GM event TC1507, and 40 % and 38 % for GM event MIR604 for maize powder levels 1 and 2, respectively.

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

For this comparative testing round ILC-EURL-GMFF-CT-02/11 participants were provided with a guidance document for the estimation of the measurement uncertainty (MU). About 53 % of participants provided information on MU in a complete and consistent manner. Despite the provision of a guidance document in this comparative testing round to assist participants with the estimation of MU, the percentage of participants who reported the MU in a correct way decreased from about 56 % in the previous comparative testing round to about 53 % in this 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  $619/2011^{(26)}$ . In case that the reported value minus the expanded uncertainty is above 0.9 % the sample would have to be reported as containing GM.

## 11. References

- 1. European Commission (2003). Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Off. J. Eur. Union* L 268: 1-23
- **2**. European Commission (2004). 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. *Off. J. Eur. Union* L 191: 1-52

- European Commission (2006). Regulation (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the Community reference laboratory for genetically modified organisms. *Off. J. Eur. Union* L 368: 99-109
- 4. ISO/IEC 17043:2010 Conformity assessment General requirements for proficiency testing
- Analytical Methods Committee (2004). GMO Proficiency Testing: Interpreting z-scores derived from log-transformed data. RSC. *AMC Technical Brief.* No. 18. December 2004
- Thompson, M., Ellison, SLR., Owen, L., Mathieson, K., Powell, J., Key, P., Wood, R., Damant, AP. (2006). Scoring in Genetically Modified Organism Proficiency Tests Based on Log-Transformed Results. *J. AOAC Int.* 89: 232-239
- Analytical Methods Committee (1989). Robust statistics How not to reject outliers Part 1. Basic Concepts. *Analyst* 114: 1359-1364
- **8.** GP41GP40/EURL Assessment of the homogeneity and stability of test items for comparative testing. EU-RL GMFF internal quality document.
- **9**. Dube, S., Qin, J., Ramakrishnan, R. (2008). Mathematical Analysis of Copy Number Variation in a DNA Sample Using Digital PCR on a Nanofluidic Device. *PLoS ONE* 3: e2876. doi:10.1371/journal.pone.0002876
- **10.** ISO Guide 34:2009 General requirements for the competence of reference material producers
- **11.** ISO 13528:2005 Statistical methods for use in proficiency testing by interlaboratory comparisons
- **12**. Thompson, M., Wood, R. (1993). The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories. *J. AOAC Int.* 76: 926-940
- **13**. Linsinger, TPJ., van der Veen, AMH., Gawlik, BM., Pauwels, J., Lamberty, A. (2004). Planning and combining of isochronous stability studies of CRMs. *Accred. Qual. Assur.* 9: 464-472
- 14. European Commission (2004). Recommendation (EC) No 2004/787 of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003. Off. J. Eur. Union L 348: 18-26
- **15**. JCGM 100:2008 Evaluation of measurement data Guide to the Expression of Uncertainty in Measurement
- **16**. EURACHEM/CITAC Guide CG4 (2000). Quantifying Uncertainty in Analytical Measurement, 2<sup>nd</sup> edition
- **17**. Analytical Methods Committee (2001). Robust statistics: a method for coping with outliers. *AMC Technical Brief.* No. 6. April 2001
- **18**. Powell, J., Owen, L. (2002). Reliability of Food Measurements: The Application of Proficiency Testing to GMO Analysis. *Accred. Qual. Ass.* 7: 392-402
- **19**. Trapmann, S., Conneely P., Contreras, M., Corbisier, P., Gancberg, D., Gioria, S., Schimmel, H., Emons, H. (2004). The Certification of Reference Materials of Dry-Mixed Maize Powder with different Mass Fractions of GA21 Roundup Ready® Maize. Certified

Reference Materials ERM<sup>®</sup>-BF414. ISBN 92-894-7953-1. EU certification report EUR 21203 EN

- 20. Trapmann, S., Conneely P., Contreras, M., Corbisier, P., Gancberg, D., Hannes, E., Gioria, S., Muñoz-Pineiro, A., Van Nyen, M., Schimmel, H., Szilagy, S., Emons, H. (2005). The Certification of Reference Materials of Dry-Mixed Maize Powder with different Mass Fractions of 1507 Maize. Certified Reference Materials ERM<sup>®</sup>-BF418. ISBN 92-894-9748-3. EU certification report EUR 21689 EN
- 21. Trapmann, S., Conneely P., Contreras, M., Corbisier, P., Gioria, S., Van Nyen, Vincent, S., M., Emons, H. (2006). The Certification of Reference Materials of Dry-Mixed Maize Powder with different Mass Fractions of MIR604 Maize. Certified Reference Materials ERM<sup>®</sup>-BF423. ISBN 92-79-01635-0. EU certification report EUR 22175 EN
- **22**. Trapmann, S. (2006). Use of Certified Reference Materials for the quantification of GMO in food and feed. ERM Application Note 4, <u>http://www.erm-crm.org/ERM products/application notes/application note 4/Documents/application note 4 english.pdf</u>
- **23**. ENGL Guidance Document (2008). Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing. <u>http://gmocrl.jrc.ec.europa.eu/doc/Min Perf Requirements Analytical methods.pdf</u>
- 24. EU-RL GMFF website. Overview of validated event-specific real-time PCR quantification methods. <u>http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm</u>
- **25**. Caprioara-Buda, M., Meyer, W., Jeynov, B., Corbisier, P., Trapmann, S., Emons, H. (2012). Evaluation of plasmid and genomic DNA calibrants used for the quantification of genetically modified organisms. *Anal. Bioanal. Chem.* 404: 29-42
- **26**. European Commission (2011). Regulation (EU) No 619/2011 of the European Parliament and of the Council of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorisation procedure is pending or the authorisation of which has expired. *Off. J. Eur. Union* L 166: 9-15

# 12. Questionnaire data

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

1. DNA extraction method?	No. of laboratories
a) ISO validated	38
b) EU-RL validated	4
c) National reference method	4
d) International literature	6
e) In-house developed and optimised	14
f) Other	28
Other of which	Most answers referred to the use of kits,
	see Question 4
Lipp et al. (2001). Eur. Food Res. Technol. 212:	1

<u>497-504.</u>

1.3. Was the DNA extraction method used within the scope of your ISO/IEC 17025 accreditation?	No. of laboratories
a) Yes	78
b) No	16

No. of laboratories
2
67
12
10
3
1
1
1

3. Sample intake (in g) for the DNA	No. of laboratories
extraction?	
a) < 0.1	3
b) 0.1-0.2	62
c) > 0.2	24
d) Other	7
Other of which	
0.4	2
0.5	1
1.0	3
2.0	1

4. DNA extraction method/kit used?	No. of laboratories
a) CTAB	39
b) CTAB-derived	16
c) Biotecon	2
d) GeneScan GENESpin	4
e) Guanidine HCl with proteinase K	3
f) Macherey Nagel Nucleospin	15
g) Promega Wizard	7
h) Qiagen DNeasy plant mini kit	8
i) TEPNEL kit	1
j) Proprietary method	0
k) Other	3
Other of which	
Modified DNeasy Blood & Tissue kit	1

R-Biopharm SureFood PREP Plant X Surefood PREP Allergen Fa. Congen

1 1

5. How was the clean-up of the DNA performed?	No. of laboratories
a) No DNA clean-up	44
b) Ethanol precipitation	15
c) Amersham MicroSpin S300	0
d) Promega Wizard DNA clean-up resin	9
e) Qiagen QIAQuick	8
f) Qiagen Genomic-Tip 20/G	1
g) Silica	4
h) Proprietary method	2
i) Other	11
Other of which	
Chloroform, isopropanol precipitation, ethanol	
washing	1
GeneElut MicroSpin	1
GeneScan GeneSpin	1
GeneScan, cleaning columns	1
INVITEK Invisorb DNA Clean up	1
Isopropanol precipitation	2
JetQuick Spin Kit GENOMED	1
Promega Wizard SV Genomic DNA purification	
system (in-house modified)	1
QIAGEN QIAmp DNA Minikit	1
Qiagen DNeasy Mini Plant Kit	1

6. How have you quantified the DNA?	No. of laboratories
a) Gel	1
b) UV spectrophotometer	34
c) Nanodrop	33
d) Fluorometer	13
e) Other	4
f) Not applicable (i.e. DNA was not quantified)	9
Other of which	
Estimation was made using qPCR	1
Gel and Fluorometer	1
NanoVue	1
qPCR	1

7. Dilution buffer?	No. of laboratories
a) TE (10 mM Tris-HCl, 1 mM EDTA)	15
b) TE 0.1X (10 mM Tris-HCl, 0.1 mM EDTA)	12
c) TE low (1 mM Tris, 0.01 mM EDTA)	3
d) Water	57

e) Other	7
Other of which	
0.5X TE	1
AE buffer from Qiagen DNeasy Mini Plant Kit	1
No dilution applied	1
TE (10 mM TrisHCl, 0,2 mM EDTA)	1
TE 0.2x (2 mM Tris-HCl, 0.2 mM EDTA)	3

8. Screening method used for GM detection?	No. of laboratories
a) Combinatory SYBR® Green qPCR Screening (CoSYPS)	1
b) In-house developed and optimised	3
c) International literature	6
d) ISO/CEN published method	9
e) National reference method	11
f) Pre-spotted plate	5
g) Qualitative PCR	5
h) Real-time PCR	47
i) SYBR® Green qPCR Screening	1
h) Other	6
Other of which	
Events non-specific screening wasn't performed	1
In-house monitor run	1
No screening method was applied as the events were known	1
Only event-specific methods	1
R-Biopharm SureFood GMO 35S/Nos/FMV Screening	1
Real-time PCR qualitative event-specific methods were performed for	1
the detection of GM events	
Real-time PCR: German screening methods (P-35S+T-nos, CTP2-	1
CP4-EPSPS, bar, 35S-pat)	
Real-time screening was also performed	1

# 8.3 Screening method used within the scope of your ISO/IEC No. of laboratories 17025 accreditation?

a) Yes	69
b) No	25

9. Principle of detection used for	No. of laboratories
screening	
a) Gel	16
b) MGB	0
c) Roche probe	0
d) SYBR® Green	3
e) Taqman probe	76
f) Other	2
Other of which	
Combination of gel and qPCR event-specific	1

methods

Real-time was also used	1	
10. Screening method used for GM	No. of laboratories	
detection		
a) Multiplex PCR	13	
b) Singleplex PCR	81	

11. Elements/targets used for screening	No. of laboratories
(P = promoter, T = terminator)	
a) bar	19
b) bla	0
c) CP4 EPSPS	12
d) cry1Ab	2
e) manA	0
f) ntpII	5
g) P35S	59
h) P-ract	1
i) P-ubiZM1	1
j) T-35S	1
k) T-nos	48
I) Other	39
Other of which	
35S-pat	5
5' flanking/CaMV	1
5' flanking/cry	1
5' flanking/insert	1
actin 1	1
CaMV	1
CTP2-CP4EPSPS	9
FMV	1
IPC	1
LB/plant	1
LY038	7
P35S-pat	1
pat	8
pFMV	1

12. Real-time PCR quantification method(s)	No. of laboratories
a) EU-RL validated method(s)	72
b) In-house developed and optimised	6
c) International literature	0
d) ISO/CEN published method(s)	4
e) National reference method(s)	3
f) Other	5
Other of which	

Eurofins GMO Quant event(s) MIR604, GA21, TC1507 (HR) kits,	1
verified by the laboratory	
GeneScan GMOQuant event NK603 Corn, Surefod GMO Bt 176 Corn	1
GeneScan GMO Quant Event MIR604 Corn; GMOQuant Event NK603	1
Corn; GMO Quant Event TC1507 Corn; GMO Quant Event GA21	
Corn	
ISO 21570 for GA21	1
ISO and EU-RL GMFF validated methods	1

12.3. Real-time PCR quantification method used within the scope of your ISO/IEC 17025 accreditation?	No. of laboratories
a) Yes	61
b) No	32

13. Real-time PCR quantification	No. of laboratories
method	
a) Multiplex PCR	0
b) Singleplex PCR	94

14. Real-time PCR instrument	No. of laboratories
a) ABI 7000	5
b) ABI 7300	4
c) ABI 7500	30
d) ABI 7700	2
e) ABI 7900HT	26
f) ABI StepOne & StepOnePlus	3
g) BioRad icycler	3
h) Corbett Rotor-Gene 6000	1
i) Roche LightCycler 480	5
j) Roche LightCycler 2.0	1
k) Stratagene Mx3000/Mx3005	6
l) Stratagene Mx4000	0
m) Other	9
Other of which	
ABI 7500 and LightCycler (from 1999)	1
ABI7300 and Roche Lightcycler 480	1
Biorad CFX96	2
BioRad İQ5	1
QIAGEN Rotor Gene Q	3
Roche LightCycler 2.0	1

15. Real-time PCR Master Mix*	No.	of
* Some laboratories used different types of real-time PCR master mix	laboratories	
a) ABI TaqMan® Universal PCR master mix	54	
b) ABI TaqMan® Universal PCR master mix, no AmpErase® UNG	15	
c) ABI TaqMan® Fast Universal PCR master mix	5	

d) ABI TaqMan® Gold with Buffer A	4
e) Eurogentec qPCR MasterMix	3
f) Sigma JumpstartTM Taq ReadyMix TM	4
g) Qiagen QuantiTect SYBR Green PCR kit	0
h) Qiagen QuantiTect Probe PCR kit	4
i) Roche FastStart TaqMan® Probe Master (Rox)	1
j) Roche FastStart Universal Probe Master (Rox)	1
k) Diagenode Universal Mastermix	2
m) Eurogentec MESA GREEN qPCR MasterMix Plus for SYBR® Assay	0
n) Eurogentec qPCR MasterMix for SYBR® Green	0
o) Eurogentec qPCR MasterMix	2
p) Fermentas Maxima™ Probe/ROX qPCR Master Mix	1
q) Fermentas MaximaTM SYBR® Green/ROX qPCR Master mix	1
r) Ampliqon RealQ PCR 2x Master Mix	0
s) Takara SYBR®Premix Ex Taq™	1
t) Takara Premix Ex Taq™	1
u) Proprietary real-time PCR master mix	1
v) Other	12
Other of which	
5 Prime MasterMix	1
ABI TaqMan® PCR Core Reagent Kit	2
Eurofins reaction mix	1
In-house made master mix	1
LightCycler 480 Probes Master	1
Master mixes for each event provided by GeneScan kits	1
Mi-Taq polymerase 0.8 U (Metabion); 1x buffer supplied with	
polymerase; MgCl <sub>2</sub> as specified in methods; 1x ROX (Invitrogen);	
500 $\mu$ M dNTPs; primers and probes as specified in methods	1
Quanta Bioscience Perfecta Sybrgreen fastmix	1
Roche: Lightcycler 480 Probes Master	2
TaqMan LightCycler	1

15.2. Number of reagents (i.e. DNA,	No. of laboratories	
primers, probe, water,) involved?		
a) 5	55	
b) 6	23	
c) 7	2	
d) 8	6	
e) Other	6	
Other of which		
3	1	
4	1	
9	1	
12	1	
13	1	
20	1	
16.1 Sample intake (in ng) per real-	No. of laboratories	
--------------------------------------	---------------------	
time PCR		
a) 0-50	9	
b) 50-100	16	
c) 100-200	31	
_d) > 200	9	
16.2 Sample intake (in ng) per real-	No. of laboratories	
time PCR		
a) 0-50	1	
b) 50-100	6	
c) 100-200	4	
d) > 200	3	
16.3 Sample intake (in ng) per real-	No. of laboratories	
time PCR		
a) 0-50	0	
b) 50-100	1	
c) 100-200	2	
d) > 200	2	
16.4 Sample intake (in ng) per real-	No. of laboratories	
time PCR		
a) 0-50	1	
b) 50-100	0	
c) 100-200	0	
d) > 200	2	
16.5 Sample intake (in ng) per real-	No. of laboratories	
time PCR		
a) 0-50	0	
b) 50-100	0	
c) 100-200	0	
d) > 200	1	

Questions 16.2 to 16.5 only had to be answered, in case of different sample intakes.

17. Number of reactions per DNA	No. of laboratories
extraction	
a) 1	1
b) 2	30
c) 3	31
d) 4	9
e) 5	5
f) 6	9
g) Other	8
Other of which	
8	3

9	3
10	1
12	1

18. Real-time PCR detection method(s)	No. of laboratories
for quantification	
a) MGB	0
b) Roche probe	0
c) Taqman probe	89
d) SYBR® Green	0
e) Other	5
Other of which	
Not applicable	3
No quantification	2

19. Real-time PCR quantification method used?	No.	of
	laboratories	
a) DNA copy number standard curve using a dilution series	32	
b) Mass/mass standard curve using a dilution series	42	
c) Delta Ct method	14	
d) Other	9	
Other of which		
2 x b) and 1 x d)	2	
and Delta Ct method	2	
Delta Ct method for MIR604	1	
Delta Ct method for NK603 and GA21	1	
DNA copy number standard curve using calibration standards		
provided by the kit	1	
MIR604 in Delta Ct, TC1507 and GA21 in standard curve	1	
Used a) copy number and c) Delta Ct method depending on event	1	

Q 20a Real-time PCR	No. of laboratories per GM event									
quantification method(s): slope(s) endogenous gene	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
-4.1 < slope < -3.6	1	1	0	0	0	3	2	1	0	0
$-3.6 \leq \text{slope} \leq -3.1$	65	4	7	5	5	63	55	6	6	41
-3.1 < slope < -2.6	5	1	1	1	1	3	6	1	1	4
Other	0	0	0	0	0	0	0	0	0	0
Not applicable	9	50	50	53	52	12	14	52	52	28
Q 20b Real-time PCR	No. of laboratories per GM event									
quantification method: GM trait gene	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
-4.1 < slope < -3.6	5	2	0	2	0	11	6	3	0	12
$-3.6 \leq \text{slope} \leq -3.1$	62	3	6	3	6	58	57	6	7	39
-3.1 < slope < -2.6	5	1	1	1	1	7	4	1	1	2
Other	2	0	0	0	0	0	0	0	0	0
Not applicable	6	49	50	52	51	6	9	51	50	23
Q 21a Real-time PCR quantification method(s):				No. o	of laborato	ries per G	M event			
R <sup>2</sup> coefficient(s) endogenous gene	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
$0.97 < R^2 < 0.98$	3	1	1	1	1	5	1	2	1	2
$0.98 \le R^2 \le 0.99$	6	0	0	0	1	7	5	0	0	4
$0.99 \le R^2 \le 1.00$	60	5	6	5	4	57	54	5	6	42
Other	2	0	0	0	0	0	1	1	0	1
Not applicable	8	49	50	52	51	11	13	51	51	28

Q 21b Real-time PCR				No	of laborate	ories per G	M event			
quantification method: R2 coefficient(s) GM trait gene	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
$0.97 < R^2 < 0.98$	4	1	1	2	1	3	1	3	0	2
$0.98 \le R^2 \le 0.99$	14	1	0	0	1	20	11	0	1	13
$0.99 \le R^2 \le 1.00$	55	4	6	4	5	53	52	7	7	38
Other	1	0	0	0	0	0	2	0	0	0
Not applicable	5	47	48	50	49	5	8	49	48	23
Q 22. Real-time PCR				No	of laborato	ories per G	M event			
quantification method(s): endogenous target DNA sequence(s)	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
Adh	18	4	4	5	3	41	35	4	6	27
Hmg	56	5	6	5	5	33	31	7	6	29
Invertase	2	0	0	1	1	2	1	0	1	2
Zein	2	0	0	0	1	3	2	3	0	2
zSSIIb	3	0	0	1	0	2	2	0	0	2
Other	2	0	1	0	1	2	2	1	0	1
Of which	Mhm		Mhm		Zm	Zmadh	Zmadh	Zm		SSIIb-3
	SSIIb-3					SSIIb-3	SSIIb-3			
Q 23. Real-time PCR quantification method(s):				No.	of laborato	ries per GN	l event			
endogenous target DNA sequence(s)	TC1507	3272	59122	Bt11	Bt176	GA21	MIR604	MON810	MON863	NK603
P35S	5	1	3	4	3	2	1	4	4	6

35S-pat	1	0	1	0	0	0	0	0	0	0
bar	0	0	0	0	2	0	0	0	0	0
bla	0	0	0	0	0	0	0	0	0	0
cry1Ab	0	0	0	0	3	0	0	0	0	0
CP4 EPSPS	0	0	0	0	1	1	0	0	0	4
hsp70 intron	0	0	0	0	0	0	0	1	0	0
manA	0	0	0	0	0	0	0	0	0	0
nptII	0	0	0	0	0	0	0	0	1	0
pat	3	0	1	2	0	0	0	0	0	0
P-ract	0	0	0	0	0	1	0	0	0	1
P-ubiZM1	0	0	0	0	0	0	0	0	0	0
<i>T-35S</i>	0	0	0	0	0	0	0	0	0	0
T-nos	1	3	1	4	1	6	5	2	4	5
1507 event-specific	82	0	0	1	0	0	0	0	0	0
3272 event-specific	0	16	0	0	0	0	0	0	0	0
59122 event-specific	0	0	18	0	0	0	0	0	0	0
Bt11 event-specific	0	0	0	16	0	0	0	0	0	0
Bt176 event-specific	0	0	0	0	12	0	0	0	0	0
GA21 event-specific	1	0	0	0	0	79	0	0	0	0
MIR604 event-specific	1	0	0	0	0	0	72	0	0	0
MON 810 event-specific	0	0	0	0	0	0	0	18	1	0
MON 863 event-specific	0	0	0	0	0	0	0	0	19	1
NK 603 event-specific	1	0	0	0	0	0	0	0	0	62
Other	0	0	0	2	2	2	0	3	0	0
Of which				Adh1 and	Cry-	CA21				
				(2)	(2)	construct (1) Construct-		<i>hsp-cry</i> (2) 5'-end		
						spec. (1)		juncuon (1)		

24. Which reference material(s)	No. of laboratories
was(were) used for calibration? *	
a) ERM-BF411 series	23
b) ERM-BF412 series	24
c) ERM-BF413 series	21
d) ERM-BF413k series	5
e) ERM-BF414 series	73
f) ERM-BF415 series	63
g) ERM-BF416 series	27
h) ERM-BF417 series	5
i) ERM-BF418 series	79
j) ERM-BF420 series	21
k) ERM-BF423 series	64
I) ERM-BF424 series	20
m) Non-modified corn leaf DNA AOCS	
0306-C	0
n) Non-modified ground corn AOCS 0406-A	0
o) Non-modified ground corn AOCS 0407-A	2
p) Ground corn GA21 AOCS 0407-B	4
q) Ground corn MIR604 AOCS 0607-A2	4
r) Dual-target plasmid(s)	3
<ul><li>s) Multiple-target plasmid(s)</li></ul>	12
t) Other	7
Other of which	
Corn standards for NK 603	1
DNA calibration standards provided by the	
kit	1
Eurofins GeneScan reference materials	1
GeneScan positive controls	1
NK603 IRMM 5 %, Sample nr 0438	1
Non-modified maize flour internally	
prepared	1
Test kits from GeneScan	1

\* Most laboratories used several reference materials

25. Which reference material(s) was(were) used for quality	No. of laboratories
control? *	
a) ERM-BF411 series	35
b) ERM-BF412 series	38
c) ERM-BF413 series	32
d) ERM-BF413k series	6
e) ERM-BF414 series	76
f) ERM-BF415 series	66
g) ERM-BF416 series	40
h) ERM-BF417 series	6
i) ERM-BF418 series	81

j) ERM-BF420 series	33
k) ERM-BF423 series	70
I) ERM-BF424 series	33
m) Non-modified corn leaf DNA AOCS 0306-C	0
n) Non-modified ground corn AOCS 0406-A	0
o) Non-modified ground corn AOCS 0407-A	2
p) Ground corn GA21 AOCS 0407-B	3
q) Ground corn MIR604 AOCS 0607-A2	3
r) Dual-target plasmid(s)	2
s) Multiple-target plasmid(s)	10
v) Other	8
Other of which	
3272 plasmid JRC	1
GeneScan positive controls	1
IRMM standard	1
Negative control ( $H_2O$ , extraction control), CRM-IRMM 411-413	1
NK603 IRMM 5 %, Sample nr 0997	1
Non-modified maize flour internally prepared	1
Positive and negative controls included in the kit	1
Samples with known GMO presence (4,2 % GA21, 0.8 % MIR604,	
0.5 % TC1507)	1

\* Most laboratories used several reference materials

Q 26. Practical LOD and				
LOQ (in %) of the GM content determination in mass/mass or DNA copy		No. of lal	ooratories	
number ratio?	LOD m/m	LOQ m/m	LOD cp/cp	LOQ cp/cp
0	1	1	2	2
0.001			1	
0.002	1			
0.003	1			
0.01	5	1	2	
0.02	5		2	
< 0.03	1			
0.03	2	1	1	
0.04	2		1	1
< 0.045	1			
0.045	1		1	
0.05	7	4	4	1
0.06				1
0.08		2		1
0.09		2	1	1
< 0.1	1			
0.1	9	23	1	7

0.2		1		
0.3		1		
0.5		1		
0.52				1
Not applicable	9	9	1	1
Not reported	50	50	79	80

## 27. Did you report the uncertainty (u) as a relative value in No. of laboratories % (i.e. does u correspond to a percentage of the reported

GM level, e.g. <i>u</i> is equal to 25 % of the reported GM level)?		
a) Yes	42	
b) No	50	
27.1. Does the uncertainty correspond to a relative	No.	of
repeatability standard deviation?	laboratories	
a) Yes	31	
b) No	17	
c) Not applicable	27	
27.2. Does the uncertainty correspond to a relative within-	No.	of
laboratory reproducibility standard deviation?	laboratories	
a) Yes	25	
b) No	21	
c) Not applicable	31	
27.4. Did you report an expanded uncertainty including a	No.	of
coverage factor?	laboratories	
a) Yes	36	
b) No	7	
c) Not applicable	24	
27.5. If applicable, please specify the coverage factor used	No.	of
(k = 1  for  a 66.67 %  confidence level, k = 2  for  a 95 %	laboratories	
confidence level, $k = 3$ for a 99 % confidence level)		
a) $k = 1$	1	
b) <i>k</i> = 2	40	
c) <i>k</i> = 3	3	

28. Did you report the uncertainty as an absolute value?	No. of laborate	ories
a) Yes	41	
b) No	49	
28.1. Does the uncertainty correpond to a repeatability	No.	of
standard deviation?	laboratories	
a) Yes	24	
b) No	17	
_c) Not applicable	29	
28.2. Does the uncertainty correspond to a within-laboratory	No.	of
reproducibility standard deviation?	laboratories	
a) Yes	14	
b) No	23	
c) Not applicable	30	
28.4. Did you report an expanded uncertainty including a	No.	of
coverage factor?	laboratories	
a) Yes	47	
b) No	9	
_c) Not applicable	30	
28.5. If applicable, please specify the coverage factor used	No.	of
(k = 1  for  a 66.67 %  confidence level, k = 2  for  a 95 %	laboratories	
confidence level, $k = 3$ for a 99 % confidence level)		
a) <i>k</i> = 1	1	
b) <i>k</i> = 2	55	
c) <i>k</i> = 3	2	

## 13. Acknowledgements

With respect to the raw material(s) used in this study we kindly acknowledge Pioneer Overseas Corporation for providing the maize event TC1507 and Syngenta Biotechnology Inc. for providing the maize events MIR604 and GA21. We sincerely thank Roberta Brustio, Stéphane Cordeil, Steven Price, Eleonora Scigliano, Pierluigi Tenuta, of the MBG Unit and EU-RL GMFF for their invaluable contributions to this fourth comparative testing round. A special thanks to Marko Maras and Karolina Kolodziej who are 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 labs listed below are kindly acknowledged for their participation.

Organisation	Department	Country	Status
Agenzia provinciale per l'ambiente di Bolzano	Laboratorio Analisi Alimenti	IT	5
Austrian Agency for Health and Food Safety (AGES)	Competence Centre Biochemistry	AT	1, 2
Agricultural Institute of Slovenia		SI	2
Agri-Food and Veterinary Authority of Singapore	Laboratory Department	SG	4
Agroscope Liebefeld-Posieux Research Station ALP	Analytics	СН	4
Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)	Laboratoire de la Santé des Végétaux	FR	1, 2
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit		DE	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	FFSD, Laboratory for GMO food	HU	1, 2
Central Agricultural Office, Food and Feed Safety Directorate	Feed Investigation NRL	HU	1, 2
Central Control and Testing Institute of Agriculture	Molecular Biology	SK	1, 2
Centre Wallon de Recherches agronomiques (CRA-W)	Valorisation des productions	BE	1, 2
Centro Nacional de Alimentación (Agencia Espaňola de seguridad alimentaria y nutricion)	Biotechnology Unit	ES	1, 2
Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL)		DE	3
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)		DE	2
Consorcio CSIC-IRTA-UAB	Molecular Genetics (OMGs)	ES	3
Crop Research Institute	Molecular Biology RLGMO	CZ	1, 2
Danish Veterinary and Food Administration	Division of Plant Diagnostics	DK	1, 2
DTU-Food, National Food Institute	Toxicology and Risk Assessment	DK	1, 2
Federal Agency for the Safety of the Food Chain		BE	5
Federal Institute for Risk Assessment (BfR)	Effect-based Analytics and Tox	DE	2
Federal Office of Public Health FOPH	Consumer Protection Directorat	СН	3
Food and Environment Research Agency (FERA)		IE	1
Food and Environment Research Agency (FERA)		UK	2
Finnish Customs Laboratory	ET2 / BIO	FI	1, 2
Food and Consumer product Safety Authority	Laboratory	NL	2
Groupe d'Etude et de contrôle des Variétés et des Semences (GEVES)	BioGEVES	FR	1, 2
INRAN - Seed Testing Station	Laboratorio Analisi Sementi	IT	2
Institute for Agricultural and Fisheries Research (ILVO)	Technology and Food Sciences	BE	1, 2
Institut für Hygiene und Umwelt	Gentechnik	DE	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	РТ	2
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta	S.C. Biotecnologie	IT	5
Istituto Zooprofilattico Sperimentale della Sardegna	Igiene degli alimenti	IT	5
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del	Food Hygiene	IT	5

Molise			
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Biotecnologie	IT	1, 2
Istituto Zooprofilattico Sperimentale delle Venezie	Microbiologia Alimentare	IT	5
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Laboratorio OGM	IT	5
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna	Reparto Genomica	т	5
Laboratoire National de santé	Food control	111	1 2
Laboratorio Arbitral Agroalimentario - MARM	OGM	FS	1 2
Laboratório Nacional Agropecuário do Rio Grande do Sul	Lab. de Biologia Molecular	BR	4
Landesamt für Umweltschutz Sachsen-Anhalt	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 für Lebensmittelchemie	DE	2
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen (LUA)	Amtliche Lebensmitteluntersuch	DE	2
LGC Limited	Molecular and Cell Biology	UK	1, 2
LGV-Office for Health and Consumer Protection	Molekularbiologie	DE	2
Lower Saxony Federal State Office for Consumer	State Food Laboratory	DE	2
		DE	2
Ministério da Agricultura, Pecuária e Abastecimento		BR	4
Ministério da Agricultura, Pecuária e Abastecimento	LANAGRO-GO	BR	4
Ministry of Finance, General Secretariat for Tax and Customs Issues, General Chemical State Laboratory (GCSL)	Food Division Athens	GR	1, 2
Ministry of Food Agriculture Ankara Provincial Control	GMO	TR	4
Ministry of Health	National Public Health Laboratory	MY	4
National Bureau of Plant Genetic Resources, New Delhi	NRC for DNA Fingerprining	IN	4
National Center of Public Health Protection	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 Food Reference Laboratory	Biotechnology and GMO Unit	TR	4
National Institute of Biology	Department of Biotechnology	SI	1, 2
National Institute of Health - Istituto Superiore di Sanità (ISS)	Vet Pub Health and Food Safety	IT	2
National Institute of Public Health in Prague	Food Safety Analyses	CZ	2
National Quality Control Laboratory of Drug and Food	Biotechnology Laboratory	ID	4
National Veterinary Research Institute	Feed Hygiene	PL	1, 2
Norwegian Veterinary Institute	Department of Diagnostic	NO	3
Plant Breeding and Acclimatization Institute – National Research Institute	GMO Controlling Laboratory	PL	2
Quality Assurance and Testing Center 3	Microbiology - GMO Laboratory	VN	4
RIKILT - Institute of Food Safety	NFA	NL	<u>1, 2</u>
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

Staatliche Betriebsgesellschaft für Umwelt und	Coschäftsboroich 6	DE	2
	Geschaltsbereicht 6		2
Staatliches Gewerbeaufsichtsamt Hildesheim	Dez. 33 Gentechnik	DE	3
State General Laboratory	GMO Laboratory	CY	1, 2
State Institute of Chemical and Veterinarian Analysis - Freiburg	Gentechnik	DE	2
State Office for Agriculture, Food Safety and Fishery Mecklenburg-Western Pomerania	Molecular Diagnostics	DE	2
State Veterinary and Food Institute Dolny Kubin	Dept. of mol. biol. analysis	SK	1, 2
Tallinn University of Technology	Departiment of Gene Technology	EE	2
Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (TLLV)	Lab for detection of GMO/foods	DE	2
Thüringer Landesanstalt für Landwirtschaft (TLL)	Untersuchungswesen	DE	3
Umweltbundesamt	Landuse and Biosafety	AT	1, 2
University of the Free state	GMO Testing Facility	ZA	4
USDA, Grain Inspection Packers Stockyards Administration, 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 only member,  $^4$  Laboratory from third country,  $^5$  Official control laboratory only

### 14. Annex 1: Invitation letter



EUROPEAN COMMISSION JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection Molecular Biology and Genomics



Ispra, 12 September 2011 JRC.DG.I.4-MBG/GVdE/dp/Ares(2011) 961974

#### NOTE FOR THE ATTENTION OF

- I. All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
- II. All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the European Union reference laboratory for genetically modified organisms.
- III. All members of the European Network of GMO Laboratories
- IV. Interested parties from third countries

Joint Research Centre · 21027 Ispra VA, Italy · TP 331 Secretariat · Phone: +39 0332 789379, Fax: +39 0332 785483

WWW: http://gmo-crl.jrc.ec.europa.eu/ + http://bgmo.jrc.ec.europa.eu + http://ihcp.jrc.ec.europa.eu

E-mail: JRC-BGMO@ec.europa.eu

Subject: Invitation to participate in the comparative test ILC-EURL-GMFF-CT-02/11

Pursuing Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) has the obligation to organise comparative testing rounds and ensure an appropriate follow-up of the results obtained.

Hereby, I would like to invite you to participate in the fourth round of comparative testing ILC-EURL-GMFF-CT-02/11. This round of comparative testing will include two test materials of maize. The participant will need to screen for 10 maize GM events namely: 3272, Bt11, Bt176, 59122, GA21, MIR 604, MON 810, MON 863, NK603, 1507. Only those GM events that have been detected will subsequently need to be quantified 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 EURL-GMFF will disclose details of the National Reference Laboratories that have been appointed under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006 to DG SANCO for the purpose of an assessment of their performance.

ISO 9001:2008 certified by



Ure

Comparative testing is organised by the EURL-GMFF in collaboration with the Institute for Reference Materials and Measurements (IRMM, Geel, BE). Registration for the fourth round of comparative testing and submission of results will be handled by IRMM. Please register electronically for the fourth comparative testing round using the following link: https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=780

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: <u>JRC-IRMM-IMEP@ec.europa.eu</u> Cc to: <u>mbg-comparative-testing@jrc.ec.europa.eu</u>

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

The deadline for registration is <u>26 September 2011</u>. Samples should be shipped during the week of <u>24 - 28 October 2011</u>. The deadline for submission of results is <u>9 December 2011</u>.

Please contact <u>JRC-IRMM-IMEP@ec.europa.eu</u> and <u>JRC-IRMM-MILC@ec.europa.eu</u> <u>ONLY</u> for difficulties with your on-line registration.

For <u>all other issues</u> (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 EURL-GMFF is looking forward to your participation.

Yours sincerely,

Guy Van den Eede Head of Molecular Biology and Genomics Unit

Copy: M. Mazzara, D. Charels, M. Maras, F. Ulberth (JRC).

2

## 15. Annex 2: Accompanying letter



EUROPEAN COMMISSION JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection Molecular Biology and Genomics



Ispra, 24 October 2011 JRCI04/MBG/GVDE/dp/Ares(2011)

#### NOTE FOR THE ATTENTION OF

- I. All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
- II. All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the European Union reference laboratory for genetically modified organisms.
- III. All members of the European Network of GMO Laboratories
- IV. Interested parties from third countries

«Address»

Subject: Participation in ILC-EURL-GMFF-CT-02/11, a comparative testing round to determine the GM content in two maize test items.

Dear «Name» «Surname»,

Thank you for participating in the ILC-EURL-GMFF-CT-02/11 comparative testing round containing two maize test items.

You will receive the test items shipped at room temperature via courier. The shipment will be carried out in the week of 24 to 28 October 2011. 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 plastic containers each containing approximately 5 g of test item
- 2. An "Acknowledgement of Reception" form
- 3. This accompanying letter

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

Joint Research Centre • 21027 Ispra VA, Italy • TP 331 Secretariat • Phone: +39 0332 789379, Fax: +39 0332 785483 E-mail: <u>JRC-BGMO@ec.europa.eu</u> WWW: http://jmo.cr.i.c.ec.europa.eu/ • http://bgmo.irc.ec.europa.eu • http://ihcp.jrc.ec.europa.eu



This round of comparative testing will include two test materials of maize. The participant will need to screen for 10 maize GM events namely: 3272, Bt11, Bt176, 59122, GA21, MIR 604, MON 810, MON 863, NK603, 1507. Only those GM events that have been detected will subsequently need to be quantified in each test material.

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:

 $mass/mass \% = \frac{mass GM [g]}{Total mass [g]} x 100 \%$  $copy/copy \% = \frac{GM DNA copy numbers [cp]}{Target taxon-specific DNA copy numbers [cp]} x 100 \%$ 

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

Please find below a preview of the results reporting page. On the reporting webpage you need to report the GM content of those GM events that have been quantified. The questionnaire contains two separate tables (one for each GM level) where the results regarding the detection of the ten GM events can be reported. These tables called 'Presence/absence of GM event' occur after Q26 of the questionnaire. Please mark with an 'x' presence/absence of the GM events under investigation.

2

3



On the results reporting page MP L1 = maize powder level 1, MP L2 = maize powder level 2

Preview reported values

I confirm I reported my results

After entering all results, please complete the questionnaire. Please remember that you will only be able to report results regarding the detection of the ten GM events in two tables (one for each GM level) occurring after Q26 in 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 you will or have already received by E-mail is intended as an aid in the laboratory. In this pdf file, items with the word '(number)' indicate that a numerical value should be provided. Pdf files of questionnaires bearing hand-written answers will not be accepted for reporting.

## Only results and answers to the questionnaire that are reported on-line on the reporting website <a href="https://web.jrc.ec.europa.eu/ilcReportingWeb">https://web.jrc.ec.europa.eu/ilcReportingWeb</a> 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 (<u>JRC-IRMM-IMEP@cc.europa.eu</u>). Check your results carefully before submission, since this is your final confirmation.

The deadline for submission of results is <u>9 December 2011</u>. It will not be possible to submit your results after the deadline.

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

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

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

Yours sincerely, (SIGNED)

Guy Van den Eede Head of Molecular Biology and Genomics Unit 4

## 16. Annex 3: Confirmation of shipment

Reference: JRC.DG.I.4-MBG/GVdE/dp/ARES(2011)1166687

#### Dear participant,

all test parcels related to the fourth round of comparative testing have left our premises on October 26<sup>th</sup>. 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:

#### XXXXXXXXXXXXXXXXXXXX

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

- Two plastic containers each containing approximately 5 g of test item
- An acknowledgement of reception form, that should be returned to the EURL-GMFF by fax (+39 0332 789333). In case you did not yet receive the test items please contact Dario PARDI (<u>Dario.PARDI@ec.europa.eu</u>; phone +39 0332 78 51 65),
- An accompanying letter entitled 'Participation in ILC-EURL-GMFF-CT-0211'

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. Items bearing a question mark icon on the right-hand side contain valuable and important information for the participant. In the reporting website clicking on the icon will give access to this information. Pdf files of questionnaires bearing hand-written answers will not be accepted. Only results and answers to the questionnaire reported on-line reporting website to the https://web.jrc.ec.europa.eu/ilcReportingWeb will be accepted.

The deadline for submission of your results is <u>9 December 2011</u>.

Please contact <u>JRC-IRMM-IMEP@ec.europa.eu</u> and <u>JRC-IRMM-MILC@ec.europa.eu</u> <u>ONLY</u> for reporting difficulties, failures or anomalies of the online system for reporting (i.e. <u>https://web.jrc.ec.europa.eu/ilcReportingWeb</u>).

For <u>all other issues</u> (communications, questions related to the content of the comparative testing round) please contact:

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

Please send me an e-mail (<u>Dario.PARDI@ec.europa.eu</u>) in case you have not received the above-mentioned documents. Thank you.

Kind Regards, Dario Pardi

Dario Pardi - Secretariat European Commission - Joint Research Centre Institute for Health and Consumer Protection Molecular Biology and Genomics Unit Via E. Fermi, 2749 I - 21027 Ispra (VA)

Phone: + 39 0332 785165 Fax: + 39 0332 786159 E-mail: Dario.PARDI@ec.europa.eu http://www.ihcp.jrc.ec.europa.eu

## 17. Annex 4: Acknowledgement of receipt FAX - Record for Quality System

We have rece	ived the following samples	In good cond	ition Yes I	No
21027	ISPRA (VA) Italy	File nb EURL-	CT-02/11	
Europea	n Commission - Joint Research	Centre - IHCP		
To : Molecu Method	lar Biology and Genomics Unit Validation / EURL-GMFF	fax:	+39 0 332 78 615	59
			Lab Co	ode:
From :				
Revision. 4				
Date: <b>R71GP6</b> Page 1/1	<b>/EURL</b> 19/0	07/2011 <b>Acknow</b>	ledgement of recept	tion
JKC.1.4 -101 v				

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 26040 EN – Joint Research Centre – Institute for Institute for Health and Consumer Protection

Title: Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604

Author(s): Diana Charels, Marko Maras, Karolina Kolodziej, Fernando Cordeiro Raposo, Inge Verbist and 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 fourth comparative testing round ILC-EURL-GMFF-CT-02/11. Participants were required to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. Any events detected were then to be quantified.

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 maize test items were produced in-house. The Food Safety and Quality Unit managed the on-line registration and submission of results.

A total of 159 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11. Ninety-three laboratories from 40 countries returned results, of which 62 were National Reference Laboratories, seven were only members of the European Network of GMO Laboratories, eight were only Official control laboratories and 16 were laboratories from third countries. Nine laboratories including one National Reference Laboratory, one European Network of GMO Laboratory and seven laboratories from third countries from third

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in mass/mass % for both maize powder levels 1 and 2 regardless of the GM event. However, a lower percentage (43 - 86 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in copy/copy %.

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

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.



