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Comparative Testing Report on the Detection and Quantification of GM Events in Mexican Tortilla and Maize Flour

Comparative testing round: ILC-EURL-GMFF-CT-01/16

European Union Reference Laboratory for Genetically Modified Food and Feed

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Joint Research Centre

EURL European Union Reference Laboratory for GM Food & Feed

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EURL
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for GM Food & Feed

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Executive Summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF), accredited under ISO/IEC 17043, organised a comparative testing (CT) round for National Reference Laboratories (NRLs) nominated under Regulation (EC) No 882/2004 (NRL/882), with voluntary participation of other official control laboratories.

Two test items were distributed: a complex food material composed of Mexican tortilla flour spiked with maize GM events 1507 and MIR162 (Test Item 1, T1) and a feed sample composed of maize flour containing maize event 40278 (Test Item 2, T2). Participants were required to identify the presence of any GM maize event(s) in T1, then quantify those event(s). Participants were also required to identify which of three given GM maize events were present in T2, and then again, quantify those event(s). The results should have been reported in GM mass/mass %.

Eighty-four participants from 37 countries participated to this CT round, including 31 NRL/882 from 24 EU Member States. Two NRL/882 only analysed T2 because the T1 matrix was out of their scope of analysis. The qualitative results reported by the NRL/882 were generally good, however, one NRL/882 failed to identify the two GM events in T1 and another NRL/882 did not test for two of the events. The majority of NRL/882 performed satisfactorily also for the quantitative results, however, 5 NRL/882 received at least one unsatisfactory z-score. All but 3 NRL/882 had quantified every GM event they found.

Among the 53 other (non-NRL/882) participants, all but two laboratories correctly identified the three events tested for, however, 17 laboratories did not test for every event. Seven participants received at least one unsatisfactory z-score and two other participants underperformed because they had reported the concentration of 1507 or MIR162 as below the LOQ. The results of this CT round emphasised the importance of the DNA extraction step of the analytical procedure and the adequate assessment of the suitability of the extracted DNA for quantitative PCR analysis.

The performance for event-specific quantification could not be evaluated for those participants that had not reported a quantitative result for the events 1507, MIR162 or 40278 .

Follow-up actions will be organised for the laboratories with an unsatisfactory outcome for one or more GM events in this CT round.

Content

Drafted by:

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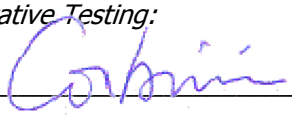
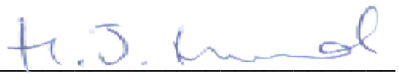
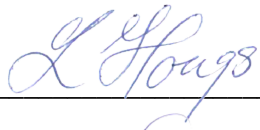
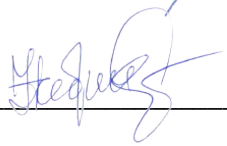
P. Corbisier 

H. Hird 

L. Hougs 

N. Papazova 

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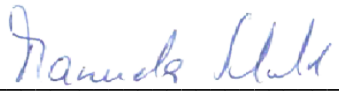
M. Schulze 

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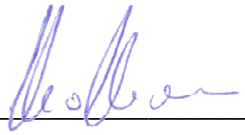
M. Mazzara (Competence group leader) 

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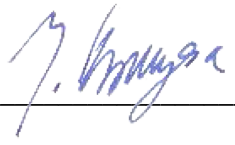
J. Kreysa (Head of Unit) 

1. Introduction

The Joint Research Centre (JRC) of the European Commission was established as European Union Reference Laboratory for GM Food and Feed (EURL GMFF) by Regulations (EC) No 1829/2003[1] and (EC) No 882/2004[2]. Regulation (EC) No 882/2004 also requires Member States to designate National Reference Laboratories (NRL/882) for each EURL to coordinate the official controls to ensure the verification of compliance with food and feed law. The EURL GMFF is tasked with the organisation of comparative testing (CT) for the NRLs to foster their correct application of the analytical methods available for these controls[2]. For this purpose, the EURL GMFF is accredited under ISO/IEC 17043[3].

Regulation (EC) No 1829/2003 established a threshold for labelling of food and feed products (0.9 %). Furthermore, Regulation (EU) No 619/2011[4] introduced a minimum performance limit (0.1 m/m %) for detecting the accidental presence, in feed, of GMOs with pending or expired authorisation status. These values are used by the Member States of the European Union in the official control of food and feed. Therefore, it is crucial that official control laboratories can accurately and reliably determine the GM content in food and feed samples.

This report summarises the results obtained in a CT round organised by the EURL GMFF. Participation in these CT rounds is mandatory for NRL/882, recommended for NRLs nominated under Regulation (EU) No 120/2014[5] (NRL/120) and open to any official control laboratory within or outside the EU. Each participant received two flour-based test items, and was required to analyse them for their GM content using routine laboratory procedures based on real-time PCR. The EURL GMFF prepares and characterises the test items, manages the online registration of participants, evaluates the results reported by the participants and assesses their performance. This activity is supported by experts from the Advisory Board for Comparative Testing.

2. Test items

The test items were prepared by the EURL GMFF from base materials that were characterised before their use (Table 1 and 2). The base materials were ground to a flour where necessary, the water content was determined by an oven drying method and the DNA extractability was determined with two common DNA extraction methods (n = 10), i.e. CTAB (100 mg sample intake) and Macherey-Nagel NucleoSpin Food (NSpin, 200 mg sample intake). The presence of GM events in the base materials was determined using event-specific pre-spotted plates[6].

From the ingredients list, the tortilla was reported to contain 27 % maize, however, it was not known if the same percentage of maize is represented in the extracted DNA and whether the maize DNA is still suitable for PCR analysis. Real-time PCR analysis revealed a 2 Cq difference in maize *hmg* between the tortilla DNA and the 100 % maize (MIR162) reference material, confirming the presence of approximately one quarter of maize DNA in the tortilla DNA extracts. It was also noted that the DNA extractability differed between the base materials used for the preparation of T1, e.g. with both extraction methods the MIR162 flour yielded three times more DNA compared to the tortilla flour; on the 1507 flour the effect was smaller and varied with the method. It is assumed that the lower extractability of the tortilla flour results from the effects of food processing. Gel electrophoresis confirmed the partial fragmentation of the tortilla DNA.

The final test items were prepared gravimetrically in accordance with ISO Guide 34[7] ('General Requirements for the Competence of Reference Material Producers'), as follows:

- The masses of GM ingredients to add were calculated in relation to the reported percentage of maize in the tortillas flour (27 %), after taking into account their water content (Table 1);
- The compound sample was manually mixed for 10 minutes, then thoroughly mixed for 60 min in a Turbula T10B mixer.

Table 1. Characteristics of the base materials used for the preparation of test item 1.

Characteristic	Mexican tortilla	1507 maize	MIR162 maize
Type of base material	Pancake-type Mexican tortilla (27 % maize), Old El Paso brand; pre-dried at room temp.	Flour from 1507 maize (purity 100 %) used to produce ERM-BF418(8)	CRM AOCS 1208-A (>998.8 g/kg MIR162 maize)(9)
Origin	Local market	IRMM	AOCS
Grinding method	Retsch ZM200	NA	NA
Water content in m/m %, mean ± SD (*n* = 10)	5.43 ± 0.93	0.14 ± 0.32	0.18 ± 0.24
DNA extractability in ng/mg[1], mean ± SD (*n* = 10)	CTAB: 0.41 ± 0.08 NSpin: 0.27 ± 0.04	CTAB: 0.55 ± 0.12 NSpin: 0.54 ± 0.09	CTAB: 1.23 ± 0.09 NSpin: 0.76 ± 0.14
GM events detected with event-specific pre-spotted plates[2]	None	1507 (+ traces of MON810 and Bt11)	MIR162
Mass used to prepare T1 (g)	1594.47	1.84	3.69
Nominal Target GM concentration in T1 (m/m %)	/	0.45	0.90

[1] Sample intake was 100 mg for CTAB, 200 mg for NucleoSpin Food (NSpin).
[2] An all-species event-specific pre-spotted plate (PSP) was used for the tortilla flour, a maize event-specific PSP for the GM materials. NA = not applicable; SD = standard deviation.

Table 2. Characteristics of test item 2.

Characteristic	Maize feed
Type of base material	Ground maize flour spiked with 40278 maize flour
Origin	Re-used test item 2 of CT 02/14 containing 40278 maize[1] (robust mean 0.66 m/m % based on data from 56 participants); see Report ILC-EURL-GMFF-CT-02/14 - Part I for details on the preparation and characterisation

[1] Maize event 40278 is included on the list of events with pending authorisations in the EU for which a technical solution for low level presence in feed is applicable(4)

The T1 mix was used to prepare 300 test items containing 5 g of flour in 30-ml bottles using a sample divider (Retsch GmbH, Haan, DE), which were then labelled with a sample number and the description "Sample T1 (Food)". Bottles of T2, which had been prepared for CT 02/14, were re-labelled with the original sample number and the description "Sample T2 (Feed, Maize)". All test items were stored at 4 °C.

Homogeneity and stability testing of T1 was performed in-house, as described in Annex 1, using event-specific quantification methods previously validated by the EURL GMFF. Material T1 was found to be homogeneous for both GM events (p-value > 0.05). The average measured MIR162 concentration (2.34 m/m %) in T1, was found to be 2.6 times higher than expected on the basis of the gravimetric preparation; this was confirmed by droplet digital PCR and may be due to the higher extractability of the MIR162 DNA compared to the DNA from the tortilla. However, as the assigned value will be calculated on the robust mean of the participants' results, the target gravimetric percentage is of no consequence for this CT exercise. From the isochronous study, it was concluded

that the test item would be sufficiently stable under ambient shipment conditions (5 % significance level).

Homogeneity and short-term stability of T2 had been previously demonstrated as part of CT 02/14. Stability (on the longer term) was re-confirmed by analysis of three extractions each from two bottles stored at 4 °C and one bottle stored at the reference temperature (-70 °C). A two-sample t-test assuming equal variances revealed the absence of a significant difference between the results obtained on bottles stored at 4 °C and -70 °C, thereby confirming the stability of the test items.

3. Tasks to be performed by participants

Participants in this CT round were required to analyse the two test items (T1 and T2) as follows:

For Test Item 1: "Food" (Mexican tortilla, pancake type):

- Identify the GM maize event(s) present in the material;
- Quantify the GM maize event(s) detected.

For Test Item 2 "Feed, Maize":

- Screen for the presence of the following three GM maize events: 1507, 40278, MIR162;
- Quantify the GM maize event(s) detected.

Quantitative results were to be reported in m/m % as outlined below:

$$\text{m/m \%} = \frac{\text{Mass GM event [g]}}{\text{Total mass species [g]}} \times 100\ \% \qquad (1)$$

Participants were reminded of the general rule that results obtained using a calibrant certified for GM mass fraction (*i.e.* a matrix CRM certified in [x] g/kg) can directly be expressed in m/m %. Results obtained using a calibrant certified for copy number ratio (*e.g.* a plasmid containing both the GM and reference gene target or some matrix CRMs) must be converted into m/m % by the participant, using their own conversion factor (to be detailed in the questionnaire); further guidance has been published by the EURL GMFF[(10)].

4. Results

4.1 Participation to CT round 01/16

On 24 March 2016, 165 laboratories were invited to participate in the CT round ILC-EURL-GMFF-CT-01/16, 85 laboratories subsequently registered for it. Eighty-four laboratories from 37 countries returned results within the reporting deadline. One laboratory did not submit any results. Table 3 shows an overview on the participation in this CT round.

The participating laboratories fell into the following assigned categories (Table 4):

- Thirty-one NRLs designated under Regulation (EC) No 882/2004 (NRL/882), representing 24 EU Member States; Ireland has delegated its NRL/882 tasks to one of the CT participants; Estonia, Malta and Latvia were not represented in this CT round;
- Twenty-two NRLs nominated only under Regulation (EU) No 120/2014 (NRL/120);
- Thirty-one official control laboratories, but not NRLs nominated under either Regulation. This category includes 12 EU laboratories and 19 laboratories from non-EU countries.

Table 3. Invitation and participation to the comparative testing round ILC-EURL-GMFF-CT-01/16.

Characteristic of the CT round	Result
Date of invitation	24 March 2016
Number of invited laboratories	165
Number of registered laboratories	85
Date of shipment of samples	12 and 13 April 2016
Deadline for result submission	24 May 2016
Registered laboratories that failed to submit their data	1
Number of participating laboratories	84

Table 4. Overview of participants by country and category.

Country	Total Participants	NRL/882	NRL/120	Non-NRL
AUSTRIA	2	2		
BELGIUM	4	3		1
BRAZIL	1			1
BULGARIA	3	1		2
CHILE	1			1
COLOMBIA	1			1
CROATIA	1	1		
CYPRUS	1	1		
CZECH REPUBLIC	1	1		
DENMARK	1	1		
FINLAND	2	1	1	
FRANCE	3	3		
GERMANY	17	1	14	2
GREECE	1	1		
HUNGARY	2	1		1
ITALY	7	1	2	4
LITHUANIA	1	1		
LUXEMBOURG	1	1		
MEXICO	1			1
NETHERLANDS	2	1	1	
PHILIPPINES	1			1
POLAND	5	2	1	2
PORTUGAL	1	1		
ROMANIA	2	1	1	
SERBIA	2			2
SINGAPORE	1			1
SLOVAKIA	1	1		
SLOVENIA	1	1		
SOUTH AFRICA	1			1
SPAIN	2	2		
SWEDEN	1	1		
SWITZERLAND	2			2
TURKEY	2			2
UKRAINE	1			1
UNITED KINGDOM	3	1	2	
UNITED STATES	1			1
VIETNAM	4			4
Total	**84**	**31**	**22**	**31**

4.2 Information on the testing provided in the questionnaire

Participants were asked to fill in an EUSurvey questionnaire on their testing methodology for T1 and T2, consisting of a number of multiple-choice questions. A total of 80 laboratories completed the questionnaire (L18, L31, L41 and L55 did not complete the questionnaire). Table 5 summarises the main answers received; Annex 2 shows all answers.

Table 5. Summary of the main answers provided in the questionnaire of CT 01/16.

Question	Test Item 1	Test Item 2
Tasks performed	Identification+quantification (93 %[1]), only identification (6 %)	Identification+quantification (78 %), only identification (20 %)
DNA extraction method	CTAB (53 %), NucleoSpin (19 %)	CTAB (54 %), NucleoSpin (19 %)
DNA purification method	None (64 %), Ethanol (15 %)	None (63 %), Ethanol (15 %)
Number of replicates	2 (68 %), 4 (15 %)	2 (68 %), 4 (16 %)
PCR inhibition tested	Delta Cq between two or more dilutions (44 %), OD ratios (33 %)	Delta Cq between two or more dilutions (45 %), OD ratios (33 %)
GM events detected	1507 (93 %), MIR162 (83 %)	40278 (83 %), none (15 %)
GM events not tested	MON87427 (60 %), 5307 (58 %)	Not applicable (78 %), 40278 (15 %)
Maize endogenous gene	*hmg* (81 %), *adh1*-134 to 136 bp (24 %)	*hmg* (64 %), *adh1*-134 to 136 bp (10 %)
Calibrant used	1507: CRM IRMM in m/m % (81 %), CRM IRMM in copy number ratio (4 %) MIR162: pure CRM AOCS (64 %), CRM IRMM in m/m % (9 %)	40278: CRM IRMM in m/m % (76 %), pure CRM AOCS (1 %)
Reason for lack of quantification	Not applicable (78 %), reagents not available (14 %)	Not applicable (78 %), reagents not available (11 %)
Reason for lack of analysis	Matrix is out of scope (1 participant), only screening methods available (1)	Reagents not available (2 participants)

[1] The percentages shown are per total number of answers received including blanks.

4.3 GM event identification

Table 6 summarises the results reported by the participants on GM event identification.

Among the NRL/882, only L29 failed to identify the events in T1, and L26 did not test for event MIR162 and 40278 because they lacked all necessary reagents.

Two (NRL/882) laboratories (L55, L84) did not analyse T1 (food being out of their scope of analysis), as agreed between the three NRL/882 within the Member state and communicated to the EURL GMFF. Two other (non-NRL) laboratories did not analyse T2 due to the lack of the necessary reagents.

A few additional GM events were identified in T1, i.e. MON810, MON863, T25 and 40278.

Table 6. GM event identification results reported by the laboratories (number of laboratories); the labcodes of NRL/882 are shown in bold.

Qualitative Results Reported	Test Item 1		Test Item 2
	1507 Maize	**MIR162 Maize**	**40278 Maize**
Present	77	69	70
Not tested	3 (L48, L61, L71)	11 (L06, L18, **L26**, L28, L30, L44, L48, L61, L63, L71, L77)	12 (L01, L06, L15, L20, L23, **L26**, L28, L30, L56, L61, L63, L82)
Tested but not detected	2 (**L29**, L79)	2 (**L29**, L64)	0
Test item not analysed	2 (**L55**, **L84**)		2 (L48, L71)

4.4 GM event quantification

4.4.1 Quantitative results reported by the participants

Of the 84 laboratories that participated to this CT round, the number of participants that submitted event-specific quantitative data for each of the GM events present in the test items is shown in Table 7. Additionally, L20 reported a value of <0.1 m/m % for 1507 in T1 (using digital PCR), L70 reported for both 1507 and MIR162 "<LOQ" in the questionnaire, and L18 reported a "larger than" value for MIR162 in T1 (>5 m/m %); these results were not included in the calculation of z-scores because the calculation macro requires the conversion of results to logarithmic values; this was outlined in the letter with the instructions for this CT round. The "below the LOQ" values, which are the results of quantitative measurements, were considered when evaluating the overall performance of the laboratories (Section 4.5), while the "larger than" value could not be evaluated because of the uncertainty on their meaning. Similarly, the performance of those laboratories that have not reported a quantitative result for one or more of the events could not be evaluated.

Table 7. Quantitative GM event-specific results reported by the laboratories.

Quantitative Results Reported	Test Item 1		Test Item 2
	1507 Maize	**MIR162 Maize**	**40278 Maize**
Number of laboratories reporting a quantitative result	73	63	66
Number of laboratories reporting the measurement uncertainty	60[a]	50[a]	55[b]
Number of laboratories reporting the coverage factor used	55	46	48

[a] Includes two laboratories (L01 and L72) reporting the uncertainty as a relative uncertainty.
[b] Includes one laboratory (L72) reporting the uncertainty as a relative uncertainty.

Furthermore, MON810 was quantified in T1 at 0.05 m/m % by L48, T25 at 0.47 m/m % by L45 and at 0.17 m/m % by L58. L29 reported a value of 0.33 m/m % for event 40278 in T1 (and had not detected 1507 and MIR162), while this laboratory also reported a value of 0.36 m/m % for this event in T2; therefore it does not appear that the results for T1 and T2 had been switched, as a value for 40278 should only have been reported for T2 as it was not present in T1. Except for the low level presence of MON810, the values reported for the other events are considered as incorrect outcomes for these analyses.

A measurement uncertainty was reported for 81 % of all measurement results, with the coverage factor reported for 74 % of the results. These percentages are similar to those in previous CT

rounds. Two laboratories returned a relative measurement uncertainty for the events quantified (in % of the quantitative value). Among the NRL/882, all but 4 laboratories (L13, L33, L65 and L73) systematically provided a measurement uncertainty for every result reported.

For calibration of the analysis for 1507, IRMM has produced a CRM certified in m/m %[8]. Three laboratories reported the use of a "CRM from IRMM certified in GM DNA copy number ratio (plasmid calibrant)", which does not exist; it is unclear which type of calibrant they used. One of these laboratories (L35) reported the application of a conversion factor of 2 to convert the results to m/m %, the other (L45) did not specify if a conversion factor had been used. Two other laboratories (L26 and L54) used a non-certified reference material expressed in copy number ratio and applied a conversion factor of 2 (among these two, L26 received an unsatisfactory z-score for 1507; see below). L20 performed digital PCR and reported a result of <0.1 m/m % without specifying a conversion factor.

For MIR162, a CRM certified for purity and expressed in m/m % is available from AOCS: the majority of laboratories used this CRM for calibration. Six laboratories have erroneously reported the use of a CRM from IRMM (which does not exist for this event), others used a non-certified reference material expressed in m/m % (L50 and L82) or used digital PCR (L20).

For 40278, the CRM from IRMM certified in GM m/m %[11] was used by all but one laboratory; L60 erroneously reported the use of a CRM from AOCS for this event, which does not exist.

4.4.2 Assigned values

The assigned values for events 1507 and MIR162 in T1, and 40278 in T2, were based on the consensus values (μ_R) from the data from participants in this CT round, calculated using robust statistics[12,13]. This approach minimises the influence of outlying values.

The expanded uncertainty (U) on the results comprises standard uncertainty (u) contributions from the characterisation of the material by the laboratories (u_{char}) and the between-test item homogeneity determined by the EURL GMFF (u_{bb})[14], and is estimated according to:

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

A coverage factor (k) of 2 was used to calculate the expanded uncertainty corresponding to a 95 % level of confidence[15]. The standard uncertainty on the characterisation (u_{char}) was calculated using the formula:

$$u_{char} = \frac{\sigma}{\sqrt{N}} \qquad (3)$$

where: σ = robust Relative Standard Deviation of the robust mean expressed in m/m %
N = number of data points

The assigned values and associated uncertainties for all GM events are reported in Table 8.

Table 8. Overview of assigned values and expanded uncertainties for the GM events in T1 and T2.

Assigned Values & Uncertainties	Test Item 1		Test Item 2
	1507 Maize	**MIR162 Maize**	**40278 Maize**
Type of assigned value	Robust mean, μ_R	Robust mean, μ_R	Robust mean, μ_R
Number of data points	73	63	66
$u_{char,\ rel}$ (%)	5.58	4.82	2.16
$u_{bb,\ rel}$ (%)	8.01	3.65	3.06
Assigned Value (m/m %)	**0.76**	**2.60**	**0.63**
Expanded Uncertainty ($U = 2*u$)	0.15	0.31	0.05

The robust means for the events spiked into the tortilla flour (T1) were 1.7 (1507) and 2.9 (MIR162) times larger than the gravimetrically calculated GM percentages, but corresponded well with the values obtained by droplet digital PCR for these events (confirmatory measurements from EURL GMFF). This discrepancy may have several causes: the maize source used for the preparation of the tortillas and the type of processing are not known (whole kernels or kernels without the embryos for example), the maize percentage reported for the tortillas is an approximate value, the spiked GMO CRMs have not undergone the processing steps used to prepare the tortillas, and the DNA extractability of the different base materials differed markedly. Industrial processing of maize for the preparation of tortillas is often performed by nixtamalisation (a dry milling process with alkali), which removes some of the maize embryos and most of the pericarp and involves cooking the kernels; maize fractions, e.g. maize pericarp, may also be added[(16)]. As these processes affect the quality of the maize genomic DNA in a way that is difficult to predict, it is not unexpected that the actual GM percentages measured in the final compound food material used for the CT round deviated from the targeted values. This phenomenon has been seen previously. Furthermore, the difference in DNA extractability between 1507 and MIR162 CRMs may explain why the deviation between the nominal target and measured GM content is larger for MIR162 than for 1507.

It is important to note that the robust mean calculated for event 40278 (0.63 m/m %) corresponds well with the value calculated for this event in the same test item in CT 02/14 (0.66 m/m %).

4.4.3 Calculation of z-scores

To evaluate laboratory performance, z-scores were calculated for the GM events quantified on the basis of the assigned value and the target standard deviation for each event (see Annex 3, formula A3.1). The target standard deviations were fixed by the Advisory Board for Comparative Testing at 0.2 for T1 and 0.15 for T2, in line with the complexity of the test item matrix, and taking into account the results of previous CT rounds. For consistency, all decimal numbers were rounded to two digits.

Table 9 summarises the performance characteristics for GM event quantification by the laboratories participating in this CT round. Detailed results per laboratory are reported in Annex 4, Tables A4.1 to A4.3 and Figures A4.1 to A4.3.

A total of 15 quantitative results, reported by 12 laboratories, resulted in an unsatisfactory z-score, the majority of which (10) were for event 1507 in T1. Among the 31 NRL participants designated under Regulation (EC) No 882/2004 ("Category a" participants, or NRL/882) 5 obtained an unsatisfactory outcome for one or more of their reported results.

Table 9. Evaluation of laboratory performance for GM event quantification through z-scores.

Laboratory Performance	Test Item 1		Test Item 2
	1507 Maize	MIR162 Maize	40278 Maize
Assigned value μ_R	0.76	2.60	0.63
Lower z-score limit (μ_R – 2.0)	0.28	0.99	0.31
Upper z-score limit (μ_R + 2.0)	1.80	6.26	1.24
Number of laboratories with a satisfactory z-score	63	60	64
Number of laboratories with an unsatisfactory z-score	10	3	2

The unusually large number of unsatisfactory z-scores for event 1507 are due to the particular data distribution for this event (displayed in Figure A4.1), which, although symmetrical, deviated from a normal distribution as it displayed accentuation around the mean (higher kurtosis) whilst at the same time containing several very extreme data points. Removal of the extreme values resulted in a normal distribution, however, without having a significant effect on the robust mean or on the number of unsatisfactory z-scores for this event. All data were therefore included in the analysis.

4.5. Overall performance of the laboratories

The overall performance of the laboratories participating in this CT round was evaluated on the basis of both the qualitative (*i.e.* the correct identification of the GM events) and the quantitative results reported. A satisfactory performance outcome was attributed to those laboratories who had correctly identified the GM event and obtained a satisfactory z-score for its quantification. The laboratories who had not tested a GM event or those who had identified the event but had not reported a quantitative value were not considered as overall satisfactorily performing. While individual laboratories may have a valid reason for not analysing a certain GM event, the overall satisfactory performance score provides an estimate of the capacity of the participants in this CT round to adequately detect and quantify each of the three GM events. The result of the evaluation is shown in Table 10. Detailed results per laboratory are reported in Annex 5, Tables A5.1 to A5.3.

A detailed analysis of the unsatisfactory results reported by a number of laboratories once more highlighted the importance of the DNA extraction step of the analytical procedure. Notably, two laboratories (L16 and L80) who obtained an unsatisfactory z-score for both events in T1 were the only two laboratories that had used Qiagen's DNeasy plant kit for DNA extraction (one other laboratory also used this kit but did not provide a quantitative result for any event). Furthermore, L29, who had not identified 1507 or MIR162 in T1 (but identified and quantified 40278), was the only laboratory that reported the use of the "Maxwell 16 Food, Feed, Seed" nucleic acid extraction system and kit from Promega. Similarly, L70 was the only laboratory who had used an SDS method (followed by Wizard resin clean-up) and reported the 1507 and MIR162 content as below the LOQ. In these cases it may be assumed that the quality (or quantity) of the extracted DNA may have compromised the results. This emphasises the importance to verify the extraction procedure for every type of sample matrix. It is further worrying that some laboratories did not assess the quality of the extracted DNA or only verified the optical density ratios ($OD_{260/280}$ and $OD_{260/230}$). As also reported in previous CT reports (*e.g.* CT 02/14, part 2), such approaches may not be sufficient for the quality assessment of DNA extracted from complex food or feed matrices.

One laboratory (L20) reported the use of digital PCR for quantification of 1507 and MIR162; quantification of MIR162 was satisfactory (the conversion factor used was not reported in the questionnaire), but they failed to quantify event 1507 in T1 (reported as <0.1 m/m %).

In other cases of unsatisfactory performance the causes were not obvious, but will be further investigated in consulation with the laboratories concerned.

In general, the results revealed a higher satisfactory performance for the NRL/882 participants compared to the other categories of participants (Table 10). Whereas most of the NRL/120 participants also performed satisfactorily, the score of the non-NRL participants reduced the overall satisfactory performance percentage considering all laboratories; this was mainly because many non-NRL had not analysed these GM events (see Table A5.3 in Annex 5).

Table 10. Overall performance characteristics for laboratories participating in comparative test ILC-EURL-GMFF-CT-01/16 (labcodes of NRL/882 are shown in bold).

Laboratory Performance	**Test Item 1**		**Test Item 2**
	1507 Maize	**MIR162 Maize**	**40278 Maize**
Laboratories with a satisfactory performance	L01, L02, L03, **L04**, L07, L08, L09, L10, **L11**, L12, **L13**, **L14**, L15, **L17**, L19, **L21**, **L22**, L23, L24, **L25**, L30, L31, **L32**, **L33**, L34, L35, L36, **L37**, **L38**, L40, L41, L42, L43, L44, L46, **L47**, L49, L51, **L52**, L53, L54, L56, L57, L58, **L59**, L60, L62, L63, **L65**, L66, L67, **L68**, **L69**, L72, **L73**, L74, **L75**, L77, **L78**, **L81**, L82, **L83**, **L85**	L01, L02, L03, **L04**, L07, L08, L09, L10, **L11**, L12, **L13**, **L14**, **L17**, L19, L20, **L21**, **L22**, L24, **L25**, L27, L31, **L32**, **L33**, L34, L35, L36, **L37**, **L38**, L39, L40, L41, L42, L43, **L45**, L46, **L47**, L49, L50, L51, **L52**, L53, L54, L58, **L59**, L60, L62, **L65**, L67, **L68**, **L69**, L72, **L73**, L74, **L76**, **L78**, L79, **L81**, L82, **L83**, **L85**	L02, L03, **L04**, L07, L08, L09, **L11**, L12, **L13**, **L17**, L19, **L21**, **L22**, L24, **L25**, L27, **L29**, L31, **L32**, **L33**, L34, L35, L36, **L37**, **L38**, L39, L40, L41, L42, L43, L44, **L45**, L46, **L47**, L49, L50, L51, **L52**, L54, **L55**, L57, L58, **L59**, L60, L64, **L65**, L66, L67, **L68**, **L69**, L70, L72, **L73**, L74, **L75**, **L76**, L77, **L78**, L79, **L80**, **L81**, **L83**, **L84**, **L85**
Laboratories that did not test for the GM event	L48, L61, L71	L06, L18, **L26**, L28, L30, L44, L48, L61, L63, L71, L77	L01, L06, L15, L20, L23, **L26**, L28, L30, L56, L61, L63, L82
Laboratories that failed to correctly identify the GM event	**L29**, L79	**L29**, L64	
Laboratories that identified but did not quantify the GM event	L06, L64	L15, L23, L57, L66, **L75**	L10, L16, L53, L62
Laboratories that reported a <LOQ result for the GM event	L20, L70	L70	
Laboratories with an unsatisfactory z-score (for quantification)	L16, L18, **L26**, L27, L28, L39, **L45**, L50, **L76**, **L80**	L16, L56, **L80**	**L14**, L18
Satisfactory performance considering all laboratories[1]	63/82 (77 %)	60/82 (73 %)	64/82 (78 %)
Satisfactory performance of NRL/882[1]	24/29 (83 %)	25/29 (89 %)	29/31 (94 %)

[1] Number of laboratories (or NRL/882) with a satisfactory performance per total number of laboratories (or NRL/882) participating to the analysis. Note that L55 and L84 (NRL/882) did not test T1 and L48 and L71 (non-NRL) did not test T2; these laboratories were excluded from the totals.

5. Conclusions

Participants in this CT round were required to analyse two test items varying in composition and complexity. The analytical tasks resembled the routine operational analysis tasks of an official control laboratory analysing a food or feed material for the presence of GMOs.

The results reported by the participants were analysed and a performance evaluation was carried out taking into account both the qualitative and the quantitative results reported and including the missing results; a failure to test or to quantify a GM event were considered unsatisfactorily in relation to the tasks of this CT round. The majority of the participants performed satisfactorily for all tasks in this CT round, *i.e.* the detection and quantification of the events 1507 and MIR162 in T1, and 40278 in T2. Only 3 laboratories failed to correctly identify both GM events in T1, and 2 other laboratories reported a value <LOQ for one or both events. Relatively more unsatisfactory z-scores were obtained for event 1507 (10) compared to MIR162 (3) and 40278 (2).

The performance was in general higher for T2 compared to T1, an observation in line with the complexity of the material to be analysed, *i.e.* T1 was a processed food material while T2 was (unprocessed) maize flour. The results emphasise the importance of the first step in the modular analytical procedure required for GMO analysis, *i.e.* the use of a suitable DNA extraction method and the adequate assessment of the suitability of the extracted DNA for quantitative PCR analysis.

All participants, and NRL/882 specifically, are reminded that it is mandatory under EU legislation to be able to identify and quantify all GM events that are authorised in the EU or for which the authorisation is pending or has expired, or to have a procedure in place to delegate such tasks to another laboratory.

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

COUNTRY	ORGANISATION	DEPARTMENT	CITY
CATEGORY[1] a			
AUSTRIA	AGES - Institute for Food Safety Vienna		Vienna
AUSTRIA	Umweltbundesamt GmbH		Vienna
BELGIUM	Scientific Institute of Public Health	PBB - GMOlab	Brussels
BELGIUM	ILVO	Technology and Food Sciences	Merelbeke
BELGIUM	Centre Wallon de Recherches Agronomiques	Valorisation des productions	Gembloux
BULGARIA	National Center of Public Health Protection	GMO unit	Sofia
CROATIA	Croatian Institute of Public Health		Zagreb
CYPRUS	State General Laboratory Laboratory	GMO and Food Allergens	Nicosia
CZECH REPUBLIC	Crop Research Institute		Prague
DENMARK	Danish Veterinary and Food Administration	Plant Diagnostics	Ringsted
FINLAND	Finnish Customs Laboratory	ET2	Espoo
FRANCE	Anses, Laboratoire de la Santé des Végétaux	Equipe OGM	Angers cedex 01
FRANCE	Service Commun des Laboratoires		Illkirch-Graffenstad
FRANCE	BioGEVES		SURGERES
GERMANY	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	Referat 503	Berlin
GREECE	General Chemical State Laboratory	A΄Chemical Service of Athens	Athens
HUNGARY	National Food Chain Safety Office		Budapest
ITALY	Istituto Zooprofilattico Lazio e Toscana	Biotechnology	Rome
LITHUANIA	National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO	Vilnius
LUXEMBOURG	Laboratoire National de Santé	food control lab	Dudelange
NETHERLANDS	RIKILT Wageningen UR		Wageningen
POLAND	National Veterinary Research Institute	Feed Hygiene	Pulawy
POLAND	Regional Laboratory of Genetically Modified Food		Tarnobrzeg
PORTUGAL	INIAV		OEIRAS
ROMANIA	Institute for Diagnosis and Animal Health	Molecular Biology and GMOs	Bucharest
SLOVAKIA	State Veterinary and Food Institute, VFI in Dolny Kubin		Dolny Kubin
SLOVENIA	National Institute of Biology		Ljubljana
SPAIN	Laboratorio Arbitral Agroalimentario LAA-MAGRAMA	OGM	Madrid
SPAIN	Centro Nacional de Alimentacíon (Agencia Española de Consumo, Seguridad Alimentaria Y Nutriciòn)	Biotechnology Unit	Madrid
SWEDEN	National Food Agency	Biology Department	Uppsala
UNITED KINGDOM	LGC		Teddington
CATEGORY b			
FINLAND	Finnish Food Safety Authority Evira		Helsinki
GERMANY	Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Amtliche Lebensmitteluntersuchung	Dresden
GERMANY	CVUA Freiburg		Freiburg
GERMANY	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei MV	Dezernat 200/PCR	Rostock
GERMANY	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GB 6, Fachbereich 63	Nossen

COUNTRY	ORGANISATION	DEPARTMENT	CITY
CATEGORY b (continued)			
GERMANY	Landeslabor Berlin-Brandenburg (LLBB)	Fb I-6	Berlin
GERMANY	LTZ Augustenberg		Karlsruhe
GERMANY	Bavarian Health and Food safety Authority (LGL)		Oberschleissheim
GERMANY	Thüringer Landesamt für Verbraucherschutz (TLV)	Lebensmittelsicherheit	Bad Langensalza
GERMANY	Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	Halle
GERMANY	LAVES	LVI-Braunschweig/Hannover	Braunschweig
GERMANY	Institut für Hygiene und Umwelt Hamburg	Gentechniküberwachungslabor	Hamburg
GERMANY	BfR	Food Safety	Berlin
GERMANY	Landeslabor Schleswig-Holstein		Neumünster
GERMANY	Hessisches Landeslabor		Kassel
ITALY	CREA-SCS	Sede di Tavazzano, Laboratorio	Tavazzano (LO)
ITALY	Istituto Superiore di Sanità ISS	DSPVSA, GMO and mycotoxin Unit	Rome
NETHERLANDS	NVWA		Wageningen
POLAND	Plant Breeding and Acclimatization Institute-NRI	GMO Controlling Laboratory	Błonie
ROMANIA	Laboratorul Central pentru Calitatea Semintelor si a Materialului Saditor Bucuresti	LEDOMG	Bucuresti
UNITED KINGDOM	SASA		Edinburgh
UNITED KINGDOM	Fera		York
CATEGORY c			
BELGIUM	FASFC	FLVVM	Melle
BRAZIL	Ministry of Agriculture, Livestock and Food Supply	LANAGRO-GO	Goiania
BULGARIA	Executive Environment Agency	LBM and GMO	Sofia
BULGARIA	Laboratory of SGS Bulgaria Ltd		Varna
CHILE	Servicio Agrícola y Ganadero	Biotechnology Laboratory	Santiago
COLOMBIA	National Insitutute of Surveillance in Food and Drugs	GMO Laboratory	Bogotá
GERMANY	Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe		Münster
GERMANY	Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	Jena
HUNGARY	BIOMI LTD		Gödöllő
ITALY	Istituto Zooprofilattico Sperimentale della Lombardia e Emilia Romagna		Brescia
ITALY	Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Laboratorio OGM	Perugia
ITALY	ARPA FVG	Pordenone	PORDENONE
ITALY	Agenzia provinciale per l'ambiente di Bolzano	Laboratorio analisi alimenti	Bolzano
MEXICO	Centro Nacional de Referencia en Detección de Organismos Genéticamente Modificados SENASICA	Subdirección de Detección de O	México
PHILIPPINES	Department of Agriculture, Bureau of Plant Industry	NPQSD-Post-Entry Quarantine	Los Baños
POLAND	Wojewodzki Inspektorat Weterynarii	Zaklad Higieny Weterynaryjnej	Opole
POLAND	Institute of Biochemistry and Biophysics PAS		Warszawa
SERBIA	A Bio Tech Lab	Laboratory for Biotechnology	Sremska Kamenica
SERBIA	SP Laboratorija A.D.	Genetical Dpt	Becej
SINGAPORE	Agri-Food & Veterinary Authority of Singapore	Microbiology Department	Singapore
SOUTH AFRICA	University of the Free State	GMO Testing Facility G2	Bloemfontin
SWITZERLAND	Agroscope - Institute for Livestock Sciences	Feed Analytics	Posieux
SWITZERLAND	Federal Food Safety and Veterinary Office FSVO	Risk Assessment Division	Bern
TURKEY	Ankara Food Control Laboratory Directorate	Biogenetic Laboratory	ANKARA
TURKEY	National Food Reference Laboratory	Biotechnology and GMO Unit	Ankara
UKRAINE	Ukrainian Laboratory of Quality and Safety of Agricultural Products (ULQSAP)		Chabany village
UNITED STATES	USDA-GIPSA	Biotechnology	Kansas City
VIETNAM	Quality Assurance and Testing Centre 3 (QUATEST 3)	Microbiology – GMO Testing Lab	Bien Hoa, Dong Nai
VIETNAM	National Institute for Food Control		Ha Noi
VIETNAM	National Institute for Food Control (NIFC)	GMO lab	Hanoi
VIETNAM	Agricultural Genetics Institute	GMO Detection	Hanoi

[1] Category a includes NRLs designated under Regulation (EC) No 882/2004; Category b includes NRLs nominated under Regulation (EU) No 120/2014; Category c includes official control laboratories from EU or non-EU countries that are not NRLs according to the Regulations mentioned above.

Annex 1: Homogeneity and stability of test items

A1.1 Homogeneity of test items

Homogeneity of test item T2 had been demonstrated previously and was reported in the final report of ILC-EURL-GMFF-CT-02/14 – Part I (see http://gmo-crl.jrc.ec.europa.eu/Comparative-Testing.html).

The assessment of the homogeneity[17] of T1 was performed by the EURL GMFF after the test item had been packed in its final form and before distribution to participants, using the following acceptance criterion:

$$s_s \leq 0.3\hat{\sigma} \qquad \text{(A1.1)}$$

Where s_s is the between-test item standard deviation as determined by a 1-way random effects ANOVA[18] and $\hat{\sigma}$ is the standard deviation for comparative testing. The value of $\hat{\sigma}$, the target standard deviation for comparative testing, was defined by the Members of the Advisory Board on the basis of the experience acquired with previous CT rounds, and set to 0.2 for T1 and 0.15 for T2[19].

If the criterion according to A1.1 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 the mean sum of squares within-test items MS_{within}. The relative between-test item standard deviation $s_{s,rel}$ is given by

$$s_{s,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \times 100\% \qquad \text{(A1.2)}$$

where: $MS_{between}$ is the mean sum of squares between test items

MS_{within} is the mean sum of squares within test items

n is the number of replicates for each sample

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

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

$$s_{s,rel} = u^*_{bb} = \frac{\frac{repeatability}{\sqrt{n}}\sqrt[4]{\frac{2}{N(n-1)}}}{\bar{y}} \times 100\% \qquad \text{(A1.3)}$$

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

Seven bottles (N = 7) were randomly selected and analysed in five replicates (n = 5). The criterion described in formula (A1.1) was fulfilled, indicating that T1 was homogeneous. The data from the

homogeneity study were also used for the estimation of the uncertainty contribution relating to the level of homogeneity of T1 (u_{bb}, see Table 8).

A1.2 Stability of test items

For T1, an isochronous short-term stability study involving two test samples with three replicates each (N = 2, n = 3), was conducted over two and four weeks at +4 °C, +18 °C and +60 °C [20].

The results did not reveal any influence of time or temperature on the stability of the test item (compared to storage at -70 °C) with regard to maize events 1507 and MIR162.

For T2, the short-term stability had been demonstrated previously and was reported in the final report of ILC-EURL-GMFF-CT-02/14 – Part I (see http://gmo-crl.jrc.ec.europa.eu/Comparative-Testing.html). As this test item had been stored at 4 °C for 1.5 years, its long-term stability was re-analysed by comparison of bottles stored at the normal storage temperature of 4 °C (N = 2, n = 3) with those stored at -70 °C, the reference temperature (N = 1 , n = 3). No significant difference in the 40278 content (two-sample t-Test, 95 % confidence interval) was measured between either storage temperature, confirming that the material had remained stable.

The test items were shipped at ambient temperature.

Annex 2: Questionnaire data

The results received from 80 laboratories were exported from the EUSurvey "Questionnaire on CT 01/16 analysis" and are tabulated below. Multiple answers were allowed for all questions, except for questions 1.8 and 2.8. The results of question 1.8 were manually calculated by grouping the answers reported under subquestions a-d according to the GM event.

T1: Please click the box that applies and answer the further questions that appear.

	Answers	Ratio
T1: GM event identification ànd quantification was performed	74	92.5%
T1: ONLY GM event identification (no quantification) was performed	5	6.25%
T1 was not analysed	2	2.5%
No Answer	0	0%

1.1. Select the DNA extraction method used for T1

	Answers	Ratio
CTAB	42	52.5%
NucleoSpin	15	18.75%
GeneSpin	4	5%
Promega Wizard	4	5%
DNeasy plant	3	3.75%
DNeasy mericon food	2	2.5%
Biotecon foodproof	4	5%
SDS	1	1.25%
Fast ID genomic DNA	1	1.25%
Maxwell 16 plant DNA	2	2.5%
Generon ion force	1	1.25%
SureFood prep advanced	2	2.5%
Other	5	6.25%
No Answer	1	1.25%

1.2. Select any additional DNA purification method used for T1.

	Answers	Ratio
a) No additional clean-up	51	63.75%
b) Additional ethanol precipitation	12	15%
c) Eurofins DNAExtractor cleaning column	4	5%
d) Promega Wizard DNA clean-up resin	5	6.25%
e) Qiagen QIAQuick	5	6.25%
f) Qiagen Genomic-Tip 20/G	0	0%
g) Other method (no need to specify)	5	6.25%
No Answer	1	1.25%

1.3. Indicate the number of replicate DNA extractions used to obtain the results.

	Answers	Ratio
1	0	0%
2	54	67.5%
3	8	10%
4	12	15%
5	2	2.5%
6	2	2.5%
>6	2	2.5%
No Answer	1	1.25%

1.4. Select the approach used to show absence of PCR inhibition.

	Answers	Ratio
a) None (no inhibition was suspected based on experience)	11	13.75%
b) We run two or more dilutions and verify if the delta Cq is as expected	35	43.75%
c) We run two of more dilutions and verify the final GM% are similar	15	18.75%
d) We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq for undiluted extract, compare this to the measured Cq	7	8.75%
e) We add an internal positive control to the reactions and check the Cq	11	13.75%
f) We verify that the amplification curves look normal	15	18.75%
g) We check that the optical density ratios (OD260/280, 260/230) are acceptable	26	32.5%
h) Other	1	1.25%
No Answer	1	1.25%

1.5. Select all GM events detected in T1.

	Answers	Ratio
None	3	3.75%
1507	74	92.5%
3272	0	0%
5307	0	0%
40278	1	1.25%
59122	0	0%
Bt11	0	0%
GA21	0	0%
MIR162	66	82.5%
MIR604	0	0%
MON810	2	2.5%
MON87427	0	0%
MON87460	0	0%
MON88017	0	0%
MON89034	0	0%
NK603	0	0%
T25	2	2.5%
No Answer	1	1.25%

1.6. Select the GM events that were NOT TESTED in T1 (i.e. presence or absence cannot be confirmed).

	Answers	Ratio
None, all events listed were tested	21	26.25%
1507	3	3.75%
3272	20	25%
5307	46	57.5%
40278	16	20%
59122	13	16.25%
Bt11	7	8.75%
GA21	9	11.25%
MIR162	12	15%
MIR604	12	15%
MON810	10	12.5%
MON87427	48	60%
MON87460	31	38.75%
MON88017	22	27.5%
MON89034	13	16.25%
NK603	16	20%
T25	17	21.25%
No Answer	1	1.25%

1.7. Select the maize endogenous target(s) used for relative quantification in T1.

	Answers	Ratio
Maize hmg	65	81.25%
Maize adh1-70 bp	1	1.25%
Maize adh1-134 to 136 bp	19	23.75%
Maize zSSIb	3	3.75%
Maize invertase (ivr)	1	1.25%
Maize zein	1	1.25%
Other	0	0%
No Answer	6	7.5%

1.8. Enter the GM event quantified in T1 and select the calibrant used for the standard curve (Note: if a conversion factor was used to convert between units, please report it under "Comments" at the end of the questionnaire).

1507	Answers	Ratio
CRM from IRMM, certified in GM mass fraction (g/kg); results in m/m %	65	81.25%
CRM from IRMM, certified in GM DNA copy number ratio (plasmid calibrant); conversion factor used to convert results to m/m %	3	3.75%
CRM from AOCS, certified for GM presence (assuming 100% purity); results in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction; results in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR); conversion factor used to convert results to m/m %	2	2.5%
No calibrant used, digital PCR done; conversion factor used to convert results to m/m %	1	1.25%
No Answer	9	11.25%

Note: Conversion factor reported for 1507 under "Comments": 2 (2 laboratory)

MIR162	Answers	Ratio
CRM from IRMM, certified in GM mass fraction (g/kg); results in m/m %	7	8.75%
CRM from IRMM, certified in GM DNA copy number ratio (plasmid calibrant); conversion factor used to convert results to m/m %	1	1.25%
CRM from AOCS, certified for GM presence (assuming 100% purity); results in m/m %	51	63.75%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction; results in m/m %	2	2.5%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR); conversion factor used to convert results to m/m %	0	0%
No calibrant used, digital PCR done; conversion factor used to convert results to m/m %	1	1.25%
No Answer	18	22.5%

Note 1: Conversion factor reported for MIR162 under "Comments": 0.5 (1 laboratory), 1 (1), 2 (1)
Note 2: Other GM events detected: MON810 (1), MON863 (1), T25 (2)

1.9. If applicable, why did you not quantify all GM events detected in T1?

	Answers	Ratio
a) Not applicable, all GM events detected were quantified	62	77.5%
b) The event-specific quantification method(s) is/are not validated in our laboratory	2	2.5%
c) Reference material, primers, probes, or other reagents were not available (in time)	11	13.75%
d) Quantitative result obtained was below the LOQ	3	3.75%
e) We tried, but our quantitative analysis failed	0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)	0	0%
g) Other reason	2	2.5%
No Answer	1	1.25%

1.10. Why did you not analyse test item 1 (mexican tortilla)?

	Answers	Ratio
a) The sample matrix is out of the scope of our laboratory	1	1.25%
b) The methods are not validated in our laboratory	0	0%
c) We could not obtain sufficient good quality DNA suitable for further analysis	0	0%
d) Reference material, primers, probes, or other reagents were not available (in time)	0	0%
e) We tried but our analysis failed	0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)	0	0%
g) Other reason	1	1.25%
No Answer	78	97.5%

T2: Please click the box that applies and answer the further questions that appear.

	Answers	Ratio
T2: GM event identification ànd quantification was performed	62	77.5%
T2: ONLY GM event identification (no quantification) was performed	16	20%
T2 was not analysed	2	2.5%
No Answer	0	0%

2.1. Select the DNA extraction method used for T2.

	Answers	Ratio
CTAB	43	53.75%
NucleoSpin	15	18.75%
GeneSpin	4	5%
Promega Wizard	4	5%
DNeasy plant	2	2.5%
DNeasy mericon food	1	1.25%
Biotecon foodproof	4	5%
SDS	1	1.25%
Fast ID genomic DNA	1	1.25%
Maxwell 16 plant DNA	2	2.5%
Generon ion force	1	1.25%
SureFood prep advanced	0	0%
Other	5	6.25%
No Answer	2	2.5%

2.2. Select any additional DNA purification method used for T2.

	Answers	Ratio
a) No additional clean-up	50	62.5%
b) Additional ethanol precipitation	12	15%
c) Eurofins DNAExtractor cleaning column	4	5%
d) Promega Wizard DNA clean-up resin	5	6.25%

e) Qiagen QIAQuick	4	5%
f) Qiagen Genomic-Tip 20/G	0	0%
g) Other method (no need to specify)	5	6.25%
No Answer	2	2.5%

2.3. Indicate the number of replicate DNA extractions used to obtain the results.

	Answers	Ratio
1	0	0%
2	54	67.5%
3	5	6.25%
4	13	16.25%
5	3	3.75%
6	2	2.5%
>6	1	1.25%
No Answer	2	2.5%

2.4. Select the approach used to show absence of PCR inhibition.

	Answers	Ratio
a) None (no inhibition was suspected based on experience)	10	12.5%
b) We run two or more dilutions and verify if the delta Cq is as expected	36	45%
c) We run two of more dilutions and verify the final GM% are similar	13	16.25%
d) We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq of undiluted extract, compare this to the measured Cq	6	7.5%
e) We add an internal positive control to the reactions and check the Cq	11	13.75%
f) We verify that the amplification curves look normal	13	16.25%
g) We check that the optical density ratios (OD260/280, 260/230) are acceptable	26	32.5%
h) Other	2	2.5%
No Answer	2	2.5%

2.5. Select the GM events detected in T2.

	Answers	Ratio
None	12	15%
1507	0	0%
40278	66	82.5%
MIR162	0	0%
No Answer	2	2.5%

2.6. Select the GM events NOT TESTED in T2 (i.e. presence or absence cannot be confirmed).

	Answers	Ratio
Not applicable, all events listed were tested	62	77.5%
1507	4	5%
40278	12	15%
MIR162	9	11.25%
No Answer	2	2.5%

2.7. Select the maize endogenous target(s) used for relative quantification.

	Answers	Ratio
Maize hmg	51	63.75%
Maize adh1-70 bp	1	1.25%
Maize adh1-134 to 136 bp	8	10%
Maize zSSIb	1	1.25%
Maize invertase (ivr)	1	1.25%
Maize zein	0	0%
Other	0	0%
No Answer	18	22.5%

2.8.a. Select the calibrant used for the 1507 standard curve, if applicable (Note: if a conversion facor was used to convert between units, please report it under "Comments" at the end of the questionnaire).

	Answers	Ratio
Not applicable	50	62.5%
CRM from IRMM, certified in GM mass fraction (g/kg); results expressed in m/m %	12	15%
CRM from IRMM, certified in GM DNA copy number ratio (plasmid calibrant); conversion factor used to convert results to m/m %	0	0%
CRM from AOCS, certified for GM presence (assuming 100% purity); results expressed in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction; results in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR); conversion factor used to convert results to m/m %	0	0%
No calibrant used, digital PCR done; conversion factor used to convert results to m/m %	0	0%
No Answer	18	22.5%

2.8.b. Select the calibrant used for the 40278 standard curve, if applicable (Note: if a conversion facor was used to convert between units, please report it under "Comments" at the end of the questionnaire).

	Answers	Ratio
Not applicable	0	0%
CRM from IRMM, certified in GM mass fraction (g/kg); results in m/m %	61	76.25%
CRM from IRMM, certified in GM DNA copy number ratio (plasmid calibrant); conversion factor used to convert results to m/m %	0	0%
CRM from AOCS, certified for GM presence (assuming 100% purity); results expressed in m/m %	1	1.25%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction; results in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR); conversion factor used to convert results to m/m %	0	0%
No calibrant used, digital PCR done; conversion factor used to convert results to m/m %	0	0%
No Answer	18	22.5%

2.8.c. Select the calibrant used for the MIR162 standard curve, if applicable (Note: if a conversion facor was used to convert between units, please report it under "Comments" at the end of the questionnaire).

	Answers	Ratio
Not applicable	51	63.75%
CRM from IRMM, certified in GM mass fraction (g/kg); results in m/m %	2	2.5%
CRM from IRMM, certified in GM DNA copy number ratio (plasmid calibrant); conversion factor used to convert results to m/m %	0	0%
CRM from AOCS, certified for GM presence (assuming 100% purity); results expressed in m/m %	9	11.25%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction; results in m/m %	0	0%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR); conversion factor used to convert results to m/m %	0	0%
No calibrant used, digital PCR done; conversion factor used to convert results to m/m %	0	0%
No Answer	18	22.5%

2.9. If applicable, why did you not quantify all GM events detected in T2?

	Answers	Ratio
a) Not applicable, all GM events detected were quantified	62	77.5%
b) The event-specific quantification method(s) is/are not validated in our laboratory	1	1.25%
c) Reference material, primers, probes, or other reagents were not available (in time)	9	11.25%
d) Quantitative result obtained was below the LOQ	1	1.25%
e) We tried, but our quantitative analysis failed	0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)	0	0%
g) Other reason	7	8.75%
No Answer	2	2.5%

2.10. Why did you not analyse test item 2 (maize feed)?

	Answers	Ratio
a) The sample matrix is out of the scope of our laboratory	0	0%
b) The methods are not validated in our laboratory	0	0%
c) We could not obtain sufficient good quality DNA suitable for further analysis	0	0%
d) Reference material, primers, probes, or other reagents were not available (in time)	2	2.5%
e) We tried but our analysis failed	0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)	0	0%
g) Other reason	0	0%
No Answer	78	97.5%

Annex 3: Performance statistics

The aim of performance statistics is to provide participants with a meaningful result that can be easily interpreted. The procedure followed for the evaluation of the participants' performance was agreed by the Members of the Advisory Board and assumes a normal distribution of the data.

The approach relies on the calculation of z-scores from $\log_{10}$-transformed data[21,22] based on the robust means[12,13] (μ_R) of the participants' results. The EURL GMFF calculated the consensus values from the participants' results taking the robust mean (μ_R) on both original and $\log_{10}$-transformed scale, taking into account the agreed standard deviation ($\hat{\sigma}$) for comparative testing, set to 0.2 for T1 and 0.15 for T2, based on previous experience.

The z-scores (z_i) for participant *i* reporting measurement result x_i are calculated in comparison to the robust mean as follows:

$$z_i = \left(\log_{10} x_i - \log_{10} \mu_R\right) / \hat{\sigma} \qquad \text{(A3.1)}$$

Annex 4: Participants' quantitative results and z-scores

The z-scores of all laboratories are reported in Tables A4.1-A4.3. For consistency, all decimal numbers were rounded to two digits. "Value" and "uncertainty" refer to the quantitative result and uncertainty as calculated and reported by the laboratory; "z-score" is calculated by the EURL GMFF (shown in bold if |z|>2.0).

Table A4.1. Performance of "Category a" laboratories (NRL/882) in comparative test ILC-EURL-GMFF-CT-01/16 (- = not available).

Laboratory Code	Test Item 1			Test Item 1			Test Item 2		
	1507 Maize (μ_R = 0.76 m/m %)			MIR162 Maize (μ_R = 2.60 m/m %)			40278 Maize (μ_R= 0.63 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L04	0.45	0.22	-1.0	2.07	0.48	-0.4	0.61	0.15	-0.1
L11	0.76	0.52	0.1	2.70	1.55	0.2	0.61	0.27	-0.1
L13	1.21	-	1.1	4.27		1.2	0.72	-	0.4
L14	0.70	0.20	0.0	3.70	0.55	0.9	0.15	0.10	**-4.1**
L17	0.78	0.24	0.2	2.09	0.63	-0.4	0.64	0.18	0.1
L21	0.88	0.31	0.5	2.62	0.92	0.1	0.72	0.25	0.4
L22	0.54	1.62	-0.6	3.33	0.99	0.6	0.70	0.21	0.3
L25	1.17	0.36	1.1	5.46	1.79	1.7	0.58	0.14	-0.2
L26	0.25	0.03	**-2.3**	-	-	-	-	-	-
L29	-	-	-	-	-	-	0.36	0.12	-1.6
L32	0.96	0.27	0.6	2.03	0.57	-0.4	0.65	0.18	0.1
L33	0.79	-	0.2	2.70	-	0.2	0.56	-	-0.3
L37	1.11	0.33	1.0	3.95	1.19	1.0	0.63	0.19	0.0
L38	0.47	0.23	-0.9	3.48	0.79	0.7	0.56	0.15	-0.3
L45	0.24	0.03	**-2.4**	3.31	0.37	0.6	0.40	0.05	-1.3
L47	0.74	0.38	0.1	3.24	0.96	0.6	0.60	0.18	-0.1
L52	0.63	0.29	-0.3	2.53	1.21	0.0	0.64	0.18	0.1
L55	-	-	-	-	-	-	0.48	0.30	-0.8
L59	0.74	0.24	0.1	3.41	1.33	0.7	0.64	0.20	0.1
L65	0.76	-	0.1	2.58	-	0.1	0.64	-	0.1
L68	0.45	0.13	-1.0	1.64	0.82	-0.9	0.38	0.18	-1.4
L69	0.50	0.18	-0.8	2.26	0.38	-0.2	0.64	0.08	0.1
L73	0.96	-	0.6	1.81	-	-0.7	0.74	-	0.5
L75	1.15	0.09	1.0	-	-	-	0.75	0.16	0.5
L76	0.22	0.16	**-2.6**	1.60	0.07	-1.0	0.53	0.07	-0.5
L78	0.56	0.17	-0.5	2.01	0.70	-0.5	0.56	0.19	-0.3
L80	3.08	1.23	**3.2**	0.56	0.20	**-3.2**	0.95	0.09	1.2
L81	0.40	0.20	-1.3	1.80	0.62	-0.7	0.51	0.20	-0.6
L83	0.80	0.17	0.2	2.05	0.34	-0.4	0.75	0.13	0.5
L84	-	-	-	-	-	-	0.66	0.20	0.2
L85	0.90	0.41	0.5	2.26	0.34	-0.2	0.78	0.11	0.7

Table A4.2. Performance of "Category b" laboratories (NRL/120) in comparative test ILC-EURL-GMFF-CT-01/16 (- = not available).

Laboratory Code	Test Item 1			Test Item 1			Test Item 2		
	1507 Maize (μ_R = 0.76 m/m %)			MIR162 Maize (μ_R = 2.60 m/m %)			40278 Maize (μ_R= 0.63 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L02	0.57	0.15	-0.5	3.03	0.51	0.4	0.59	0.07	-0.2
L08	0.69	0.30	-0.1	2.72	0.89	0.2	0.55	0.14	-0.4
L09	1.15	0.53	1.0	3.39	1.56	0.7	0.70	0.32	0.3
L12	1.54	0.21	1.7	3.04	0.38	0.4	0.56	0.11	-0.3
L23	0.89	-	0.5	-	-	-	-	-	-
L24	0.58	0.20	-0.5	2.04	0.82	-0.4	0.56	0.30	-0.3
L27	2.05	0.30	**2.3**	3.22	0.25	0.6	0.63	0.10	0.0
L34	0.54	0.06	-0.6	2.85	0.55	0.3	0.66	0.06	0.2
L35	0.58	0.20	-0.5	1.65	0.48	-0.9	0.70	0.09	0.3
L39	1.91	0.30	**2.1**	6.37	0.78	2.0	0.69	0.07	0.3
L42	0.35	0.08	-1.6	2.13	0.38	-0.3	0.60	0.08	-0.1
L49	0.92	0.17	0.5	2.82	0.42	0.3	0.56	0.13	-0.3
L50	0.10	0.10	**-4.3**	1.50	0.20	-1.1	0.70	0.10	0.3
L51	1.04	0.21	0.8	2.33	0.11	-0.1	1.14	0.20	1.8
L53	0.75	0.25	0.1	3.46	0.25	0.7	-	-	-
L54	0.84	0.06	0.4	2.18	0.02	-0.3	0.68	0.07	0.3
L57	0.34	0.02	-1.6	-	-	-	0.66	0.04	0.2
L58	0.41	0.18	-1.2	2.84	1.27	0.3	0.72	0.13	0.4
L60	0.45	0.13	-1.0	3.95	1.20	1.0	0.70	0.20	0.3
L66	0.93	0.29	0.6	< 0.10	-	-	0.61	0.20	-0.1
L70	(<LOQ)[1]	-	-	(<LOQ)[1]	-	-	0.56	-	-0.3
L74	1.11	0.27	1.0	3.83	0.87	0.9	0.73	0.09	0.5

[1] Reported in the questionnaire

Table A4.3. Performance of "Category c" laboratories (non-NRL) in comparative test ILC-EURL-GMFF-CT-01/16 (- = not available).

Laboratory Code	Test Item 1			Test Item 1			Test Item 2		
	1507 Maize (μ_R = 0.76 m/m %)			MIR162 Maize (μ_R = 2.60 m/m %)			40278 Maize (μ_R= 0.63 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L01	0.31	23.00	-1.8	1.16	23.00	-1.7	-	-	-
L03	0.70	0.20	0.0	2.40	0.60	-0.1	0.60	0.30	-0.1
L07	0.94	-	0.6	2.13	-	-0.3	0.51	-	-0.6
L10	0.32	0.03	-1.7	2.15	0.12	-0.3	-	-	-
L15	0.89	-	0.5	0.10	-	-	-	-	-
L16	11.44	0.70	**6.0**	0.83	-	**-2.4**	-	-	-
L18	0.27	0.12	**-2.1**	5.00	-	-	1.70	0.50	**2.9**
L19	0.98	0.43	0.7	2.53	0.55	0.0	0.52	0.13	-0.5
L20	< 0.10	-	-	3.18	-	0.5	-	-	-
L28	2.88	-	**3.0**	-	-	-	-	-	-
L30	0.50	0.31	-0.8	-	-	-	-	-	-
L31	0.44	-	-1.1	2.59	-	0.1	0.61	-	-0.1
L36	1.32	0.58	1.3	3.69	0.80	0.9	0.44	0.12	-1.0
L40	0.93	-	0.6	3.15	-	0.5	0.78	-	0.7
L41	0.83	0.13	0.3	1.80	0.30	-0.7	0.47	0.12	-0.8
L43	1.68	-	1.9	3.41	-	0.7	0.63	-	0.0
L44	0.43	0.21	-1.1	-	-	-	0.96	0.90	1.3
L46	0.92	0.24	0.5	1.26	-	-1.5	0.77	-	0.6
L56	0.88	-	0.5	0.55	-	**-3.3**	-	-	-
L62	1.09	0.30	0.9	4.86	0.30	1.5	-	-	-
L63	0.65	0.44	-0.2	-	-	-	-	-	-
L64	-	-	-	-	-	-	0.60	0.81	-0.1
L67	1.01	-	0.8	1.58	-	-1.0	0.57	-	-0.3
L72	0.60	25.16	-0.4	1.55	19.43	-1.0	0.40	27.34	-1.3
L77	0.50	0.15	-0.8	-	-	-	0.75	0.21	0.5
L79	-	-	-	1.36	0.28	-1.3	0.57	0.48	-0.3
L82	0.51	0.30	-0.7	3.97	0.53	1.0	-	-	-

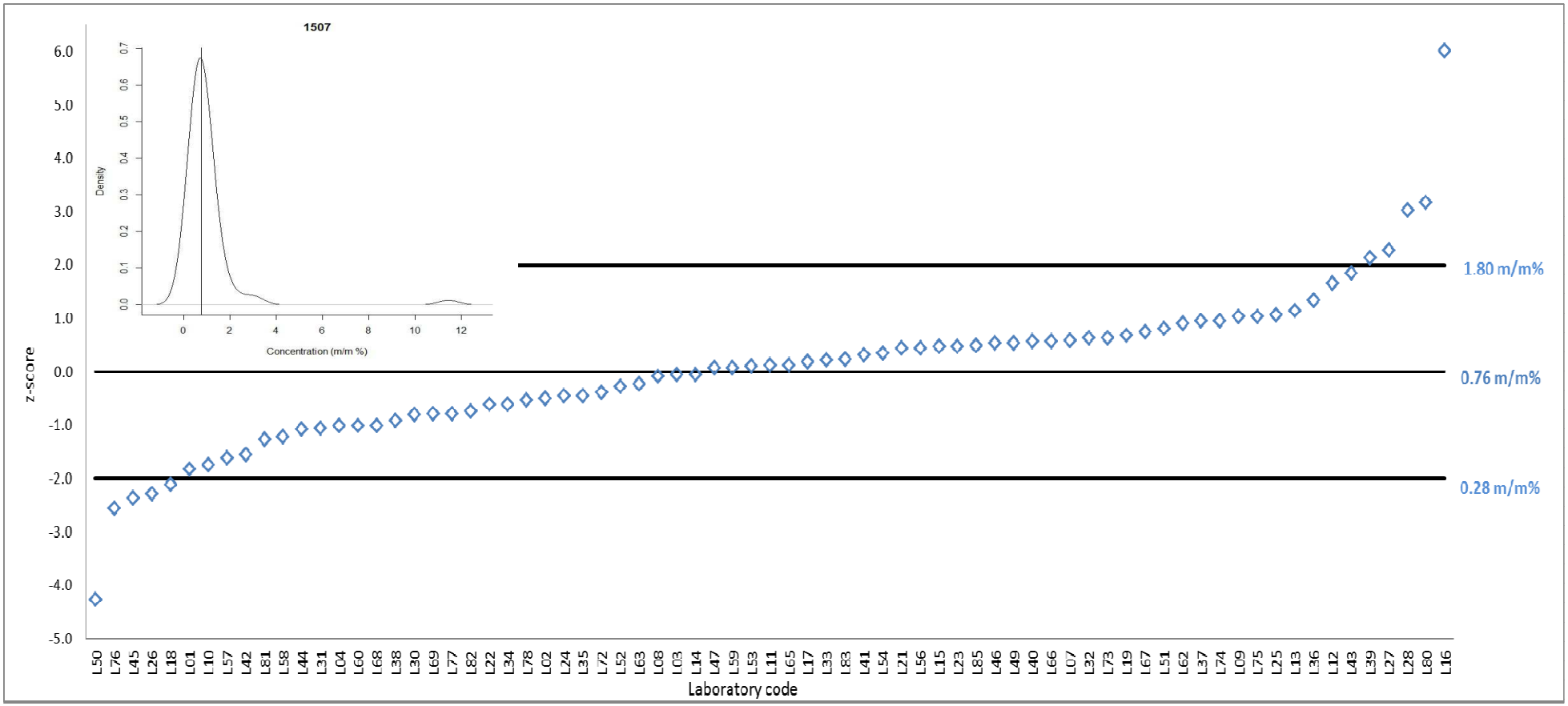

Figure A4.1. Z-scores for maize event 1507 in Test Item 1 on the basis of a robust mean of 0.76 m/m % (◊) and density plot (inset; vertical line corresponds to robust mean).

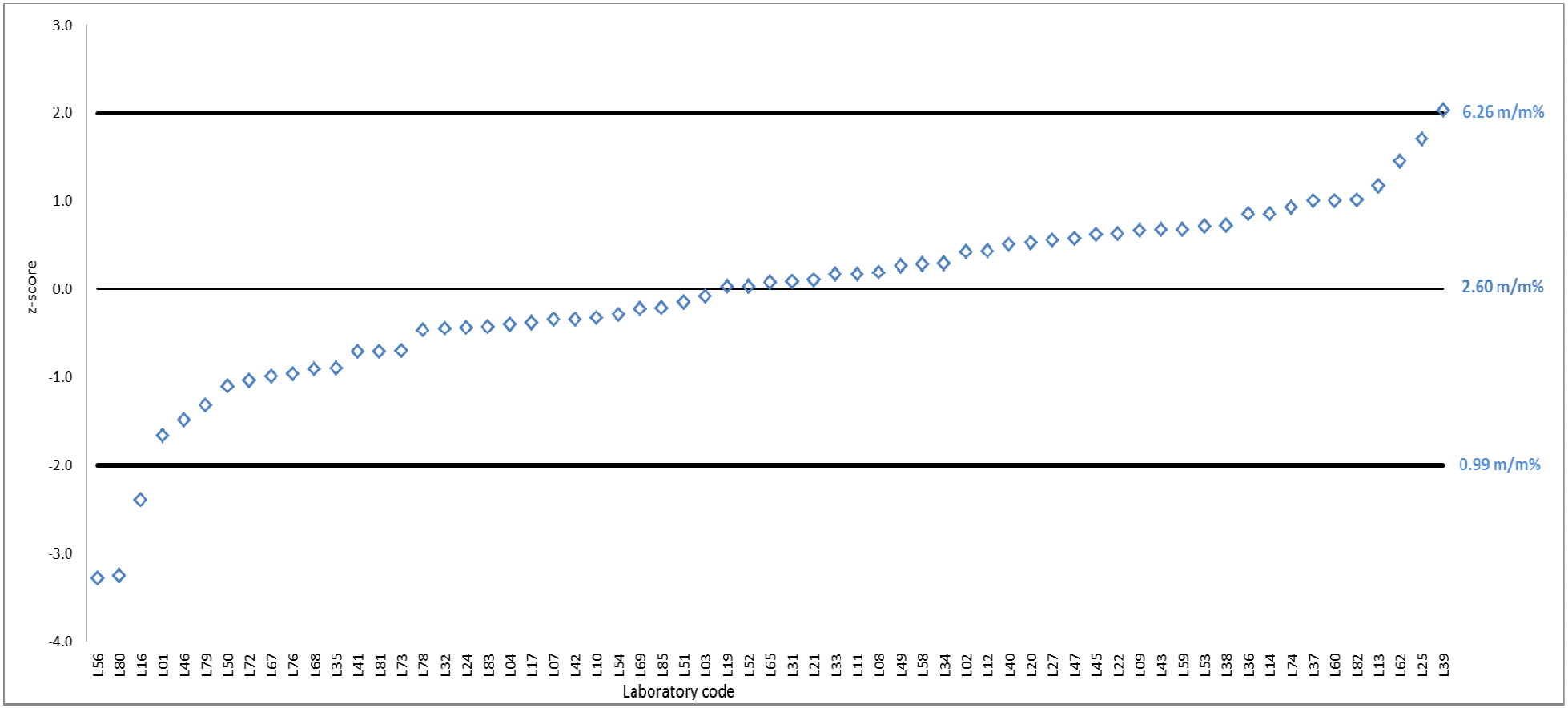

Figure A4.2. Z-scores for maize event MIR162 in Test Item 1 on the basis of a robust mean of 2.60 m/m % (◊).

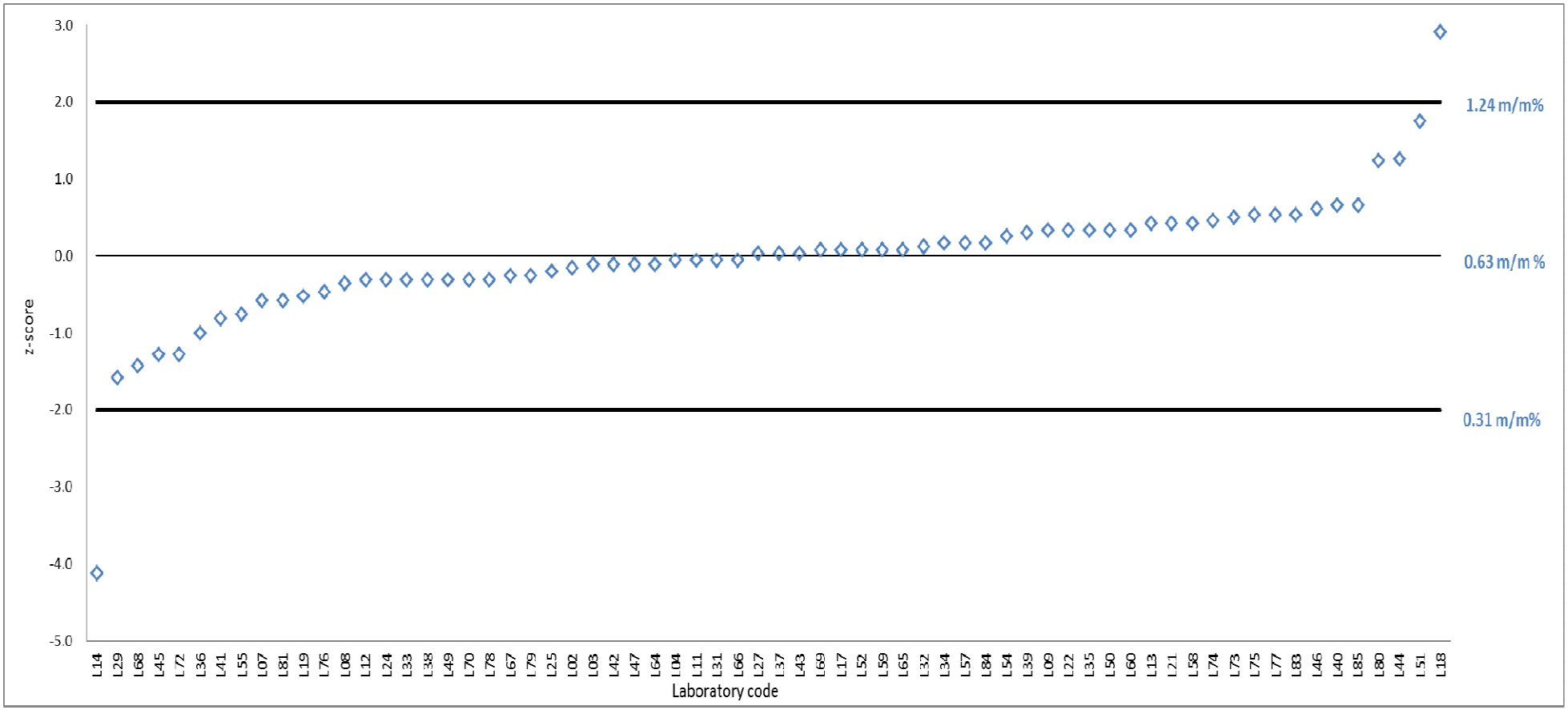

Figure A4.3. Z-scores for maize event 40278 in Test Item 2 on the basis of the assigned value of 0.63 m/m % (◊).

Annex 5: Summary of participants' performance

The performance for detection and quantification of the three GM events in the test items provided is summarised for all participants in the Tables A5.1-A5.3; the results are shown per category of participants. The row "Satisfactory" is the summing up of the participants who correctly identified the GM event or provided an acceptable quantitative result; "Incorrect" includes those participants that failed to identify the GM event or provided an unacceptable quantitative result ("<LOQ" is also considered unsatisfactory); all incorrect results are shown in bold.

Table A5.1. Performance of "Category a" laboratories (NRL/882) in comparative test ILC-EURL-GMFF-CT-01/16 (X = identified; - = not identified; NA = event not analysed).

Laboratory Code	GM identification			GM quantification		
	T1		T2	T1		T2
	1507	MIR162	40278	1507	MIR162	40278
L04	X	X	X	-1.0	-0.4	-0.1
L11	X	X	X	0.1	0.2	-0.1
L13	X	X	X	1.1	1.2	0.4
L14	X	X	X	0.0	0.9	**-4.1**
L17	X	X	X	0.2	-0.4	0.1
L21	X	X	X	0.5	0.1	0.4
L22	X	X	X	-0.6	0.6	0.3
L25	X	X	X	1.1	1.7	-0.2
L26	X	NA	NA	**-2.3**	NA	NA[3]
L29	**-**	**-**	X	NA	NA	-1.6
L32	X	X	X	0.6	-0.4	0.1
L33	X	X	X	0.2	0.2	-0.3
L37	X	X	X	1.0	1.0	0.0
L38	X	X	X	-0.9	0.7	-0.3
L45	X	X	X	**-2.4**	0.6	-1.3
L47	X	X	X	0.1	0.6	-0.1
L52	X	X	X	-0.3	0.0	0.1
L55	Not tested		X[1]	Not tested		-0.8
L59	X	X	X	0.1	0.7	0.1
L65	X	X	X	0.1	0.1	0.1
L68	X	X	X	-1.0	-0.9	-1.4
L69	X	X	X	-0.8	-0.2	0.1
L73	X	X	X	0.6	-0.7	0.5
L75	X	X	X	1.0	NA[2]	0.5
L76	X	X	X	**-2.6**	-1.0	-0.5
L78	X	X	X	-0.5	-0.5	-0.3
L80	X	X	X	**3.2**	**-3.2**	1.2
L81	X	X	X	-1.3	-0.7	-0.6
L83	X	X	X	0.2	-0.4	0.5
L84	Not tested		X	Not tested		0.2
L85	X	X	X	0.5	-0.2	0.7
Satisfactory	**28**	**27**	**30**	**24**	**25**	**29**
Incorrect	**1**	**1**	**0**	**4**	**1**	**1**
Event not analysed	**0**	**1**	**1**	**1**	**3**	**1**
Sample not analysed	**2**	**2**	**0**	**2**	**2**	**0**

[1] GM identification result was inferred from the quantification result reported (questionnaire not returned).

[2] Reason for lack of analysis: "Reference material, primers, probes, or other reagents were not available (in time)."

[3] Reason for lack of analysis: "Other reason."

Table A5.2. Performance of "Category b" laboratories (NRL/120) in comparative test ILC-EURL-GMFF-CT-01/16 (X = identified; - = not identified; NA = event not analysed).

Laboratory Code	GM identification			GM quantification		
	T1		T2	T1		T2
	1507	MIR162	40278	1507	MIR162	40278
L02	X	X	X	-0.5	0.4	-0.2
L08	X	X	X	-0.1	0.2	-0.4
L09	X	X	X	1.0	0.7	0.3
L12	X	X	X	1.7	0.4	-0.3
L23	X	X	NA	0.5	NA	NA
L24	X	X	X	-0.5	-0.4	-0.3
L27	X	X	X	**2.3**	0.6	0.0
L34	X	X	X	-0.6	0.3	0.2
L35	X	X	X	-0.5	-0.9	0.3
L39	X	X	X	**2.1**	2.0	0.3
L42	X	X	X	-1.6	-0.3	-0.1
L49	X	X	X	0.5	0.3	-0.3
L50	X	X	X	**-4.3**	-1.1	0.3
L51	X	X	X	0.8	-0.1	1.8
L53	X	X	X	0.1	0.7	NA[2]
L54	X	X	X	0.4	-0.3	0.3
L57	X	X	X	-1.6	NA[2]	0.2
L58	X	X	X	-1.2	0.3	0.4
L60	X	X	X	-1.0	1.0	0.3
L66	X	X	X	0.6	NA[2]	-0.1
L70	X	X	X	**<LOQ**	**<LOQ**	-0.3
L74	X	X	X	1.0	0.9	0.5
Satisfactory	**22**	**22**	**21**	**18**	**18**	**20**
Incorrect	**0**	**0**	**0**	**4**	**1**	**0**
Event not analysed	**0**	**0**	**1**	**0**	**3**	**2**
Sample not analysed	**0**	**0**	**0**	**0**	**0**	**0**

[2] Reason for lack of analysis: "Reference material, primers, probes, or other reagents were not available (in time)."

Table A5.3. Performance of "Category c" laboratories (non-NRL) in comparative test ILC-EURL-GMFF-CT-01/16 (X = identified; - = not identified; NA = event not analysed).

Laboratory Code	GM identification			GM quantification		
	T1		T2	T1		T2
	1507	MIR162	40278	1507	MIR162	40278
L01	X	X	NA	-1.8	-1.7	NA[2]
L03	X	X	X	0.0	-0.1	-0.1
L06	X	NA	NA	NA[2]	NA[2]	NA[2]
L07	X	X	X	0.6	-0.3	-0.6
L10	X	X	X	-1.7	-0.3	NA[2]
L15	X	X	NA	0.5	NA[4]	NA[3]
L16	X	X	X	**6.0**	**-2.4**	NA[2]
L18	X	NA[1]	X	**-2.1**	NA	**2.9**
L19	X	X	X	0.7	0.0	-0.5
L20	X	X	NA	**<LOQ**	0.5	NA[3]
L28	X	NA	NA	**3.0**	NA	NA[3]
L30	X	NA	NA	-0.8	NA[2]	NA[2]
L31	X	X	X	-1.1	0.1	-0.1
L36	X	X	X	1.3	0.9	-1.0
L40	X	X	X	0.6	0.5	0.7
L41	X	X	X	0.3	-0.7	-0.8
L43	X	X	X	1.9	0.7	0.0
L44	X	NA	X	-1.1	NA	1.3
L46	X	X	X	0.5	-1.5	0.6
L48	NA	NA	Not tested[2]	NA[2]	NA[2]	Not tested[2]
L56	X	X	NA	0.5	**-3.3**	NA[3]
L61	NA	NA	NA	NA[3]	NA[3]	NA[3]
L62	X	X	X	0.9	1.5	NA[4]
L63	X	NA	NA	-0.2	NA[2]	NA[2]
L64	X	**-**	X	NA[4]	NA[4]	-0.1
L67	X	X	X	0.8	-1.0	-0.3
L71	NA	NA	Not tested[2]	NA[2]	NA[2]	Not tested[2]
L72	X	X	X	-0.4	-1.0	-1.3
L77	X	NA	X	-0.8	NA	0.5
L79	**-**	X	X	NA	-1.3	-0.3
L82	X	X	NA	-0.7	1.0	NA
Satisfactory	**27**	**20**	**19**	**21**	**17**	**15**
Incorrect	**1**	**1**	**0**	**4**	**2**	**1**
Event not analysed	**3**	**10**	**10**	**6**	**12**	**13**
Sample not analysed	**0**	**0**	**2**	**0**	**0**	**2**

[1] GM identification result was inferred from the lack of quantification result reported (questionnaire not returned).

[2] Reason for lack of analysis: "Reference material, primers, probes, or other reagents were not available (in time)."

[3] Reason for lack of analysis: "Other reason."

[4] Reason for lack of analysis: "The method(s) are not validated in our laboratory."

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