JRC TECHNICAL REPORT

Comparative Testing Report on the Detection and Quantification of GM Events in Rice Noodles and Soybean Flour

Comparative testing round: ILC–EURL–GMFF–CT–01/15

European Union Reference Laboratory for Genetically Modified Food and Feed

2015

Abstract

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Two test items were distributed: a complex food material composed of rice noodles and soybean GM event 356043 (Test Item 1, T1) and a sample composed of soybean flour containing event 68416 (Test Item 2, T2). Participants were requested to perform species screening, then to detect and identify the GM event in T1, and to screen for the presence of any of three given GM soybean events in T2. Any GM event detected then had to be quantified. Participants had to report the results in GM mass/mass %.

Seventy-four laboratories from 34 countries registered for this CT round, of which sixty-six actually participated. All but one of the 59 laboratories that tested for the presence of event 356043 in T1 by real-time PCR correctly identified it. For T2, all but one of the 56 laboratories that tested for event 68416 correctly identified it. Six and 10 laboratories, however, did not screen T1 and T2, respectively, for the GM event present in these products.

Fifty-eight laboratories returned quantitative test results for one or both GM events using event-specific quantitative real-time PCR. The EURL GMFF calculated the robust means (μR) for soybean event 356043 in T1 (N = 51) and soybean event 68416 in T2 (N = 49). Z-scores were determined for the participants' results, based on the robust means and the target standard deviations agreed by the Advisory Board of Comparative Testing. Quantification of soybean event 356043 in T1 resulted in a satisfactory performance ($|z| \leq 2.0$) for all, but two laboratories (96 %). For soybean event 68416 in T2, all laboratories that had provided a quantitative result obtained a satisfactory z-score. Follow-up actions will be organised for the two laboratories that received an unsatisfactory performance score for soybean event 356043.

Furthermore it has to be mentioned that a large proportion (>20 %) of the 66 participants in this CT round did not test for one or for both GM events present in the samples, hence their performance for analysis of these events could not be evaluated.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

European Union Reference Laboratory
for GM Food & Feed

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Executive Summary

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Content

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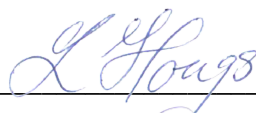
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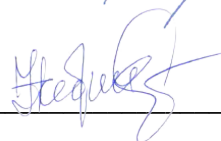
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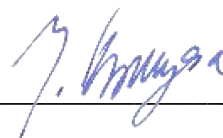*Scientific and technical approval:*

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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 Regulation (EC) No 1829/2003[1]. The EURL GMFF is also mandated by Regulation (EC) No 882/2004[2].

Article 32 of Regulation (EC) No 882/2004 tasks the EURLs with the organisation of comparative testing (CT) for National Reference Laboratories (NRLs), designated under Regulation (EC) No 882/2004 and an appropriate follow-up of such testing. The EURL GMFF is accredited under ISO/IEC 17043[3] to organise CT rounds. The aim of this activity is 'to contribute to a high quality and uniformity of analytical results'[2]. Article 12 of the said Regulation requires that the designated NRLs should be accredited under ISO/IEC 17025 on 'General Requirements for the Competence of Testing and Calibration Laboratories' and 17025-accredited laboratories must prove their competence, e.g. by taking part in comparative testing.

Regulations (EC) No 1829/2003 and (EU) No 619/2011[4] establish a threshold for labelling of food and feed products (0.9 %) and a minimum required performance limit (0.1 m/m %) for detecting low level presence of listed Genetically Modified Organisms (GMOs) in feed, respectively. As these values are used by the Member States of the European Union in the official control of food and feed, an accurate and reliable determination of the GM content is of paramount importance.

The EURL GMFF organised a comparative testing round for NRLs designated under Regulation (EC) No 882/2004 (NRL/882), whose participation was mandatory. Participation was also highly recommended for NRLs nominated under Regulation (EU) No 120/2014[5] (NRL/120) and open and free of charge for any official control laboratory. Two test items were prepared by the EURL GMFF and these were shipped to the registered participants in plastic containers containing approximately 5 g of powder. The EURL GMFF managed the on-line laboratory registration and the submission of results and was responsible for their evaluation. This activity was supported by an Advisory Board for CT. The CT round meets the requirements of ISO/IEC 17043.

This report summarises the results obtained in the 11th CT round organised by the EURL GMFF.

2. Test items

Two test items were produced in-house by the EURL GMFF: Test Item 1 (T1) consisted of the same *Rice noodles Level 2* material, containing soybean event 356043, already used in CT 02/13; T2 was composed of ground soybean containing soybean event 68416.

T1 was produced in 2013 and consisted mainly of ground rice noodles (97.9 m/m %), to which ground non-GM soybean and 356043 soybean (provided by IRMM, Geel, Belgium) were added up to a final soybean content of 2.1 % (Table 1). The GM content in the final material was determined as 1.35 m/m %, based on the robust mean of the data from 48 laboratories, as reported in the final report of this CT round (available from http://gmo-crl.jrc.ec.europa.eu/comparative-testing.html). EURL GMFF re-tested and confirmed the GM content in this material before shipment of the test items for CT round 01/15.

T2 was prepared from ground 68416 soybean flour received from IRMM, which was identical to the pure 68416 soybean flour used to prepare the CRM series for this event (ERM-BF432, available from IRMM). An oven-drying method was used for determining the remaining water content in the 68416 flour and in non-GM soybean flour, ground by EURL GMFF using an Ultra Centrifugal Mill ZM200

(Retsch GmbH, DE). The extractability of the DNA from the base materials was verified in 10 independent replicates using both the CTAB method (100 mg sample intake) and the Macherey-Nagel NucleoSpin method (200 mg sample intake). Extracted DNA (in a final volume of 100 µL for both methods) was quantified with Picogreen in a VersaFluor Fluorometer. The results showed a comparable DNA extractability from both materials using CTAB extraction, whereas with NucleoSpin almost five times less DNA was extracted from the 68416 flour compared to the non-GM soybean flour.

Four CTAB DNA extracts were randomly chosen from the 10 replicates for each base material and were assessed for the presence of inhibitors. Inhibition tests on the DNA from the non-GM soybean and 68416 soybean were done using the validated *le1* reference gene system QT-TAX-GM-002 (http://gmo-crl.jrc.ec.europa.eu/gmomethods), using 200 ng DNA in 25 µL, in line with the validated quantitative PCR (qPCR) method for 68416 soybean QT-EVE-GM-013. No inhibition was detected. The DNA extracts (100 ng in 50 µL) were furthermore assessed for the presence of GM events or species-specific DNA other than those relevant to the present comparative testing round, using ABI pre-spotted plates[6]. No other GM events or other species were identified in the conventional soybean and in 68416 soybean flour.

The final test item T2 was gravimetrically prepared in accordance with ISO Guide 34[7] ('General Requirements for the Competence of Reference Material Producers'), as follows:

- The nominal mass fractions of the GM material were produced by mixing the two flour base materials, 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. Composition of test items.

Test item	Base materials	Water content (m/m %)	Mass (g)
Test Item 1[a]	Noodles flour	6.55	2307.1
	Conventional non-GM soybean	11.24	48.83
	356043 soybean	1.89	0.67
	Total	-	2356.60
Test Item 2	Conventional non-GM soybean	14.58	1594.0
	68416 soybean	1.11	5.51
	Total	-	1599.51

[a] This material was already prepared in 2013 for use in CT 02/13.

The T1 mix was already bottled in 2013. From the T2 mix, 300 test items of 5 g were prepared in 30-ml bottles using a sample divider (Retsch GmbH, Haan, DE). Bottles were labelled with sample number and sample description (T1: "Food"; T2: "Soybean flour") and stored at 4 °C.

Homogeneity and stability testing of T1 has been performed in-house in 2013-2014. For T2, this was done as part of this CT round. Homogeneity was assessed on 7 samples per test item, analysed in 5 replicates each. Short-term stability was assessed on two bottles per test item stored at 4 °C, 18 °C and 60 °C over a period of 2 and 4 weeks, then three DNA extracts per condition were analysed. Analysis was done using the event-specific quantification methods validated by the EURL GMFF. The T2 material was found to be homogeneous for the GM event (p-value > 0.05). From the isochronous

study, it was concluded that the test item would be sufficiently stable under the shipment conditions foreseen (5 % significance level). Details on the tests performed are given in Annex 1.

3. Tasks to be performed by participants

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

For Test Item 1: "Food":

- Perform species identification (maize, soybean, oilseed rape and rice);
- Screen for the presence of GM events;
- Quantify the event(s) detected.

For Test Item 2:

- Screen for the presence of the following three soybean GM events:
  - Soybean 68416, A5547 and MON 87705;
- Quantify the event(s) detected.

Participants had to report the quantitative results in m/m % as outlined below:

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

Participants were made aware 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) needed to be converted into m/m % by the participant, using a conversion factor of his choice (to be detailed in the questionnaire); further guidance has been published by the EURL GMFF[8].

4. Results

In March 2015, a total of 181 laboratories were invited to participate in the CT round ILC-EURL-GMFF-CT-01/15 and 74 laboratories from 34 countries registered for it. Sixty-six laboratories returned results within the deadline of reporting. Table 2 shows an overview on the participation in this CT round.

The participating laboratories fell into the following assigned categories (Table 3):

a) Thirty-one NRLs designated under Regulation (EC) No 882/2004 (NRL/882);
b) Seventeen NRLs nominated <u>only</u> under Regulation (EU) No 120/2014 (NRL/120;
c) Eighteen official control laboratories, but not NRLs nominated under either Regulation mentioned. This category included 10 EU laboratories and 13 laboratories from non-EU countries.

Table 2. Participation in the comparative testing round ILC-EURL-GMFF-CT-01/15.

Date of invitation[a]	19 March 2015
Date of shipment of samples[a]	7+8 April 2015
Deadline results submission[a]	21 May 2015
Number of invited laboratories	181
Number of registered laboratories	74
Registered laboratories that failed to submit their data	L05, L11, L15, L16, L17, L45, L57, L63
Number of participating laboratories	66
Laboratories submitting only qualitative data (GM identification)	L01, L02, L23, L25, L41, L60, L73, L74
Number of laboratories with quantitative data (GM quantification)	58[b]

[a] The official letters used for communication with the (potential) participants are shown in the Annexes.
[b] This includes L48 who provided quantitative data for 9 GM (maize, soybean, rice) events, but not for those that should have been detected.

Table 3. Overview of participants per country and category.

Country	Number of participants	NRL/882 Cat. (a)	NRL/120 Cat. (b)	Non-NRL Cat. (c)
EU				
AUSTRIA	2	2		
BELGIUM	4	3		1
BULGARIA	1	0		1
CROATIA	1	1		
CYPRUS	1	1		
CZECH REPUBLIC	1	1		
DENMARK	1	1		
FINLAND	2	1	1	
FRANCE	2	2		
GERMANY	14	1	11	2
GREECE	1	1		
HUNGARY	2	1		1
ITALY	5	1	2	2
LATVIA	1	1		
LITHUANIA	1	1		
LUXEMBOURG	1	1		
NETHERLANDS	2	1	1	
POLAND	4	3		1
ROMANIA	1	1		
SLOVAKIA	2	2		
SLOVENIA	1	1		
SPAIN	2	2		
SWEDEN	1	1		
UNITED KINGDOM	3	1	2	
Total EU	56	31	17	8
Non-EU				
COLOMBIA	1			1
INDIA	1			1
MEXICO	1			1
SERBIA	2			2
SINGAPORE	1			1
SWITZERLAND	2			2
TURKEY	1			1
VIETNAM	1			1
Total non-EU	10			10
Total	**66**	**31**	**17**	**18**

4.1 Information on the testing provided in the questionnaire

Participants were requested to fill in a questionnaire consisting of 10 main questions on the testing approach used when analysing the test items. Table 4 summarises the main answers received; Annex 2 shows all answers.

On average, laboratories prepared two DNA extracts per test item using either a CTAB method or a commercial kit, both without additional clean-up. Quantitative analysis was done with real-time PCR using the EURL-validated methods from the GMOMETHODS database. *Le1* was used as endogenous taxon-specific reference gene for soybean. The CRMs from IRMM were used for calibration of the measurements, and the results were expressed in m/m % without the need for use of calculation factors to convert results expressed in copies to mass (which is to be expected for a homozygous crop such as soybean). In line with the tasks requested, a three-step approach (screening, then event-specific identification, then quantification) was used for T1. The same approach was also used for T2 by 1/3 of laboratories, while 41 % followed a two-step approach, i.e. event-specific identification, then quantification.

Table 4. Summary of information provided in the questionnaire.

Subject of Question	Question Number	Test Item	Main Answers
DNA extraction method	Q2	T1	CTAB (53 %) Commercial kit (41 %), mainly NucleoSpin (14 %)
		T2	CTAB (47 %) Commercial kit (42 %), mainly NucleoSpin (17 %)
		T1	No DNA clean-up (62 %) Commercial spin column (17 %)
		T2	No DNA clean-up (64 %) Commercial spin column (17 %)
Number of DNA extracts analysed	Q3	T1	2 extracts (71 %) 4 extracts (11 %)
		T2	2 extracts (73 %) 4 extracts (11 %)
General approach of analysis	Q4	T1	Three-step (Screening-Identification-Quantification; 73 %) Two-step (Identification-Quantification; 14 %)
		T2	Two-step (Identification-Quantification; 41 %) Three-step (Screening-Identification-Quantification; 36 %)
Real-time PCR instrument used	Q5	T1 & T2	ABI (74 %, mainly 7500 & 7900)
Digital PCR instrument used	Q6	T1 & T2	None (100 %)
Event-specific methods used	Q7	T1 & T2	EURL GMOMETHODS database (91 %)
Endogenous target DNA sequences used	Q8	T1	Soybean *le1* (100 %)
		T2	Soybean *le1* (100 %)
Reference material used	Q9	T1	CRM from IRMM (90%), mainly ERM-BF425 (80%) Data expressed in m/m% without conversion (94%)
		T2	CRM from IRMM (92%), mainly ERM-BF432 (90%) Data expressed in m/m% without conversion (96%)
Measurement uncertainty approach	Q10	T1 & T2	Calculated from repeatability (63 %), from reproducibility (27 %)

4.2 Species identification

Nearly all laboratories reported the presence of soybean and rice in T1, and the absence of maize and oilseed rape (Table 5). Two laboratories reported that rice was absent, despite the flour consisting of

almost 98 % rice noodles. One participant (L48) reported the presence of every species, and the presence of many GM events, results that are not considered reliable.

Table 5. Results (in number of laboratories) of species identification in test item T1.

Species Identification	Test Item 1			
	Maize	**Oilseed Rape**	**Soybean**	**Rice**
Present	4	3	63	61
Absent	60	57	0	2
Not tested	2	6	3	3

4.3 GM event identification

The questionnaire included tables for each test item for reporting the presence or absence of the GM events tested, with specification of the approach used to determine this (by GM screening and/or event-specific GM analysis). For T1, all EU-authorised GM events and the pending authorisations (falling under Regulation (EU) No 619/2011 for feed) were listed (one table per plant species). When a participant had determined the absence of the species in the first screening tests (Section 4.2), he/she could tick the "No GM [species] events tested" button (species referring to maize, soybean, or oilseed rape), without having to tick a button for every specific GM event of that species. For T2, the table only listed the three GM soybean events to be tested.

Table 6 summarises the results reported by the participants regarding GM event identification. In both test items the correct GM events were identified by the majority of the 66 participants, based on event-specific qualitative analysis or screening. All but one laboratory (98 %) that tested for 356043 soybean and 68416 soybean in T1 and T2, respectively, reported the presence of the respective event. However, 6 (9 %, T1) and 10 (15 %, T2) laboratories did not test for these events. Surprisingly, one laboratory (L23) reported the absence of soybean event 356043 in T1 by event-specific PCR.

In CT 02/13, which included the same test material (T1 = Level 2 test material of CT 02/13), 8 laboratories (11 %) that performed qualitative analysis for GM events did not identify 356043 soybean and another 14 laboratories did not perform any GM event-specific analyses (only species identification). Of the 8 laboratories that failed to identify event 356043 in CT 02/13, two laboratories also participated in CT 01/15; they identified the correct event in the current CT. Soybean event 356043 was authorised in 2012 in the EU, while soybean event 68416 is one of the newer GM events for which the EU authorisation is still pending, and which is listed on the EU register of GM events falling under Regulation (EU) No 619/2011[4], providing a technical solution for its low level presence on the (feed) market.

Table 6. Results (number of laboratories) of GM event identification in test items T1 and T2.

GM Event Identification	Test Item 1	Test Item 2		
	Soybean 356043	Soybean 68416	Soybean A5547	Soybean MON 87705
Present by screening	18	12	2	0
Present by event-specific PCR	57	51	1	1
Absent by screening	0	0	14	13
Absent by event-specific PCR	1	1	51	42
Not tested	6	10	4	10

4.4 GM event quantification

4.4.1 Quantitative results reported by the participants

Of the 66 laboratories that participated to this CT round, 58 participants submitted event-specific quantitative data for one or more GM events (Table 2). A number of laboratories only quantified either 356043 in T1 or 68416 in T2, and three laboratories reported semi-quantitative values for one of the events (below 0.1 m/m %; one laboratory reported "above 0.1 m/m %" without further quantification). A total of 51 quantitative values were obtained for event 356043 in T1 and 49 for event 68416 in T2. Among the 31 NRL/882 participants (category a) in this CT, only 24 provided quantitative data for 356043 soybean in T1, and 25 for 68416 soybean in T2; five NRL/882 participants provided no quantitative data at all[1].

A few additional GM events were quantified. L28 reported a false positive measurement result (0.5 m/m %) for oilseed rape event T45 in T1, and L48 (already mentioned in Section 4.2) reported quantitative values (ranging from 0.08 to 0.86 m/m %) for nine GM events in T1, not including the correct event.

Measurement uncertainties were reported for 83 % of measurement results, although a coverage factor was only reported for 74 % of the results. One laboratory (L26) returned relative measurement uncertainties for both GM events (in % of the quantitative value).

4.4.2 Calculation of consensus values

The consensus values (μ_R) for the data from participants in the CT round for the two GM events present in the samples were calculated using robust statistics[9,10]. This approach minimises the influence of outlying values.

The expanded uncertainty (U) on the results obtained comprises standard uncertainty (u) contributions from the characterisation of the material (u_{char}) and the between-test item homogeneity (u_{bb})[11], and is estimated according to:

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[1] This figure includes one NRL/882 from France for which these analyses are not within the scope of their activities, as agreed amongst the three French NRL/882 and approved by DG SANTE.

$$U = k\sqrt{u_{char}^2 + u_{bb}^2} \tag{2}$$

A coverage factor (k) of 2 was used to calculate the expanded uncertainty corresponding to a 95 % level of confidence[12]. The standard uncertainty on the characterisation (u_{char}) was calculated using the formula:

$$u_{char} = \frac{\sigma}{\sqrt{N}} \tag{3}$$

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

N = number of data points

The robust means (μ_R) for data on the non-transformed scale, and associated uncertainties, as calculated by the EURL GMFF, are reported in Table 7. The μ_R calculated for soybean event 356043 in T1 of this CT round is comparable to the value (1.35 m/m %) calculated for the same material in CT 02/13.

Table 7. Overview of robust means (μ_R) and expanded uncertainties for soybean events 356043 and 68416 in test items T1 and T2, respectively.

Test Item	GM Event	Results Expressed in m/m %		
		N	μ_R	U
T1	356043 soybean	51	1.34	0.18
T2	68416 soybean	49	0.46	0.06

4.4.3 Performance of the laboratories

To evaluate laboratory performance, z-scores were calculated for both test items on the basis of the consensus values determined from the data (see Annex 3, formula A3.1). Based on the experience in previous CT rounds and taking into account the results of previous CTs, the target standard deviation for this CT was 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. For consistency, all decimal numbers were rounded to two digits. Detailed results are reported in Annex 4, Tables A4.1 to A4.3 and Figures A4.1 and A4.2.

Two laboratories received a z-score outside the acceptable range (i.e. $|z| \geq 2.0$) for soybean event 356043 in T1. While all other results were within the range of 0.68 – 2.9 m/m %, these two laboratories reported the 356043 soybean content as 0.17 (L36) and 0.28 m/m % (L09). Both laboratories used a CTAB-based extraction method (with 2 replicates), the proper calibrant, and the EURL reference method for this event, so it is unclear why the final result obtained was that low.

All laboratories performed satisfactory for the quantification of soybean event 68416 in T2. While the latter result (all z-scores satisfactory) contradicts with the principles of the z-score approach, which dictates that 5 % of absolute z-scores are ≥ 2.0, the absence of any unsatisfactory z-score is the result of applying a σ (*a priori* set to 0.15) which is larger than the actual standard deviation of the participants' results (0.09 % in this case).

Table 8. Performance of laboratories in comparative test ILC-EURL-GMFF-CT-01/15 for quantification of soybean events 356043 and 68416.

Test Item	GM Event	Satisfactory z-score	Unsatisfactory z-score
T1	356043 soybean	L03, L06, L07, L08, L10, L12, L13, L14, L18, L19, L20, L21, L22, L24, L26, L27, L28, L29, L30, L31, L32, L33, L34, L35, L37, L38, L39, L40, L43, L46, L47, L49, L50, L51, L52, L53, L54, L55, L56, L58, L59, L61, L62, L64, L65, L67, L68, L69, L72	L09, L36
T2	68416 soybean	L03, L04, L06, L07, L09, L10, L12, L13, L14, L18, L19, L20, L22, L26 L27, L28, L29, L30, L31, L33, L34, L35, L36, L38, L39, L40, L42, L43, L44, L46, L49, L50, L51, L52, L54, L55, L56, L58, L59, L61, L64, L65, L66, L67, L68, L69, L70, L71, L72	

4.4.4 Laboratories not providing a quantitative result

A large proportion (>20 %) of the 66 participants in this CT round did not test for one or for both GM events, hence their performance for analysis of these events could not be evaluated. A few of these participants provided a semi-quantitative result in the form of a value "below" or even "above" a threshold value. Table 9 lists the participants that failed to perform quantification of the GM events identified in the test items, which was one of the requested tasks in this CT round.

Table 9. Participants to comparative test ILC-EURL-GMFF-CT-01/15 that failed to quantify soybean events 356043 and/or 68416

Test Item	GM Event	No Quantitative Result Submitted	Semi-quantitative result provided
T1	356043 soybean	L02, L04, L23, L25, L41, L44, L48, L60, L66, L70, L71, L73, L74	L01, L42
T2	68416 soybean	L01, L02, L08, L21, L23, L24, L25, L32, L37, L41, L47, L48, L53, L60, L73, L74	L62

The current results are comparable to those obtained in CT 02/13 for the same test material (T1). In CT 02/13, 8 laboratories (11 %) reporting the presence of GM material failed to identify event 356043 and 57 out of 63 participants (89 %) that had identified the event also provided a quantitative value. In the current CT, 7 laboratories (11 %) failed to identify the correct event and 89 % of those that did identify the event (51 out of 57 laboratories) also provided a quantitative result. In most of these cases of not providing data, the laboratories had not implemented the method to detect and/or quantify this soybean event. And even while 356043 soybean is uncommon on the market (if commercialised at all), official control laboratories should have the capabilities and competence to identify the event, and if needed, to quantify it. In the current CT, two NRL/882 laboratories (L04 and L71) failed to test for the presence of the event, and a total of nine NRL/882 participants did not quantify this event.

5. Conclusions

Participants in this CT round were requested to analyse two test items varying in composition and complexity. For test item T1, the requested analytical tasks were comparable to what would be done in an official control laboratory as part of a routine analysis of an unknown food material: screening for the presence of plant species of which a fraction could potentially consist of (authorised and non-authorised) GM events, identification of the GM events, and quantification of those events that were identified. The T1 matrix consisted of 97.9 % rice noodles and 2.1 % soybean, of which 1.4 % was 356043 soybean. The same test material was already used in a previous CT round (CT 02/13). Test item T2 was composed of a more uniform matrix (ground soybean) and contained 0.5 % 68416 soybean.

The species present in the T1 matrix, and the 356043 soybean event, were correctly identified by the majority of participants. The evaluation of the quantitative results for this event resulted in two laboratories receiving an unsatisfactory z-score for quantification of soybean event 356043. Also in CT 02/13, two participants performed unsatisfactory for this event; these participants obtained a satisfactory performance in the current CT. Furthermore, 9 laboratories were unable to identify the event in CT 02/13 and a total of 25 laboratories or 29 % (compared to 15 laboratories or 23 % in the current CT round) failed to provide a quantitative result. The results reveal a general improvement of the capability and performance of laboratories to test for the presence of soybean event 356043.

In T2, all but one participant (98 %) that tested for the soybean event 68416 correctly identified it, and 91 % of these also quantified the event. The quantitative results received for soybean event 68416 were satisfactory for all these participants.

The participants that have not reported quantitative results for some or both GM events to be tested are advised to implement the corresponding event-specific methods in their laboratories and make sure the resources are available for their analysis. Specifically NRL/882 are reminded that it is imperative 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.

Acknowledgements

COUNTRY	ORGANISATION	DEPARTMENT	CITY
	CATEGORY[1] a		
AT	Umweltbundesamt GmbH	Landuse & Biosafety	Vienna
AT	Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES)		Vienna
BE	Centre Wallon de Recherches Agronomiques	Valorisation des Productions	Gembloux
BE	Scientific Institute of Public Health	PBB	Brussels
BE	Institute for Agricultural and Fisheries Research	Technology and Food - PI	Merelbeke
CY	State General Laboratory	GMO & Allergens Laboratory	Nicosia
CZ	Crop Research Institute		Prague
DE	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit		Berlin
DK	Danish Veterinary and Food Administration	Plant diagnostics	Ringsted
ES	Laboratorio Arbitral Agroalimentario, LAA-MAGRAMA	OGM	Madrid
ES	Centro Nacional de Alimentación (Agencia Española de Consumo Seguridad Alimentaria y Nutrición	Biotechnology Unit	Madrid
FI	Finnish Customs Laboratory		Espoo
FR	BioGEVES		Surgeres
FR	Service Commun des Laboratoires		Illkirch-Graffenstad
GR	Ministry of Finance, General Chemical State Laboratory	A' Chemical Service of Athens	Athens
HR	Croatian National Institute of Public Health		Zagreb
HU	National Food Chain Safety Office		Budapest
IT	Istituto Zooprofilattico Sperimentale Delle Regioni Lazio e Toscana	Stuttutura di Biotecnologie	Rome
LT	National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO	Vilnius
LU	Laboratoire National de Santé	Food Control	Dudelange
LV	Institute of Food Safety, Animal Health and Environment	Virology	Riga
NL	RIKILT Wageningen UR		Wageningen
PL	Instytut Zootechniki PIB KLP Pracownia w Szczecinie		Szczecin
PL	National Veterinary Research Institute	Feed Hygiene	Pulawy
PL	Regional Laboratory of Genetically Modified Food		Tarnobrzeg
RO	Institute for Diagnosis and Animal Health	Molecular Biology and GMO	Bucharest
SE	National Food Agency		Uppsala
SI	National Institute of Biology		Ljubljana
SK	State Veterinary and Food Institute		Dolny Kubin
SK	Central Control and Testing Institute in Agriculture	Dptm. of Molecular Biology	Bratislava
UK	LGC		Teddington

COUNTRY	ORGANISATION	DEPARTMENT	CITY
	CATEGORY b		
DE	LUFA Speyer		Speyer
DE	LTZ Augustenberg		Karlsruhe
DE	LALLF MV	Dezernat 200, PCR	Rostock
DE	Institute for Hygiene and Environment		Hamburg
DE	Bavarian Health and Food Safety Authority (LGL)		Oberschleissheim
DE	CVUA Freiburg	GMO	Freiburg
DE	Landesamt für Verbraucherschutz Saarland	D5	Saarbrücken
DE	Landeslabor Berlin-Brandenburg	Fachbereich I-6	Berlin
DE	Landeslabor Schleswig-Holstein		Neumünster
DE	LAVES - Food- and Veterinary Institute Braunschweig/Hannover	FB12	Braunschweig
DE	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GB 6, Fachbereich 63	Nossen
FI	Finnish Food Safety Authority		Helsinki
IT	Istituto Superiore Di Sanità	DSPVSA	Rome
IT	CRA-SCS	Sede di Tavazzano, Laboratorio	Tavazzano (LO)
NL	Netherlands Food and Consumer Product Safety Authority (NVWA)	Consument en Veiligheid	Wageningen
PL	Institute of Biochemistry and Biophysics PAS		Warszawa
UK	Fera		York
UK	Scottish Government	SASA	Edinburgh
	CATEGORY c		
BE	Federal Laboratory for Food Safety Melle	Department of GMO	Melle
BG	SGS Bulgaria Ltd	Laboratory of SGS Bulgaria	Varna
CH	Agroscope, Institute for Livestock Sciences		Posieux
CH	Federal Food Safety and Veterinary Office FSVO	Risk Assessment Division	Bern
CO	National Institute for Food and Drug Surveillance - INVIMA	OLCC	Bogotá
DE	CVUA Westfalen		Arnsberg
DE	Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	Jena
HU	BIOMI Ltd		Gödöllő
IN	National Bureau of Plant Genetic Resources	Division of Genomic Resources	New Delhi
IT	Istituto Zooprofilattico Sperimentale Del Piemonte, Liguria e Valle D'Aosta	S.C. Biotecnologia	Torino
IT	Istituto Zooprofilattico Sperimentale Umbria e Marche	GMO laboratory	Perugia
MX	SENASICA	CNRDOGM	Tecámac
RS	Laboratory of biotechnology		Sremska Kamenica
RS	SP Laboratorija A.D.	Genetical and physico-chemical	Bečej
SG	Agri-Food and Veterinary Authority of Singapore	Veterinary Public Health Lab	Singapore
TR	National Food Reference Laboratory	Biotechnology and GMO Unit	Ankara
VN	Agricultural Genetics Institute	GMO Detection Laboratory	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 T1 has been demonstrated as part of CT 02/13. The assessment of the homogeneity[13] of T2 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:

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

Where s_s is the between-test item standard deviation as determined by a 1-way random effects ANOVA[14] 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[15].

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

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

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:

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

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

For each group of test items 7 bottles ($N = 7$) were randomly selected and analysed in five-fold replicates ($n = 5$). The criterion described in formula (A1.1) was fulfilled in all cases, indicating that all groups of test items were homogeneous. The data from the homogeneity study were also used for the estimation of the uncertainty contribution related to the level of homogeneity of test items.

A1.2 Stability of test items

An isochronous short-term stability study involving two test samples per test item with three replicates each ($N = 2$, $n = 3$), was conducted over two and four weeks at +4 °C, +18 °C and +60 °C [16].

For T1, the short-term stability was confirmed as part of the test item characterisation tests for CT 02/13 and the material was confirmed to have remained stable on the long-term. For T2, 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 soybean event 68416. The test items were therefore shipped at ambient temperature. Within the time period of this comparative study, the test materials were considered sufficiently stable.

Annex 2: Questionnaire data

Note: The answers are shown as reported by the participants. Answers with zero reported results were in most cases omitted from the tables below.

Q1. Which species and GM events were, or were not, identified in the test items?

Q1.1. Species Identification in T1	Number of Laboratories			
	Maize	Oilseed Rape	Soybean	Rice
Present	4	3	63	61
Absent	60	57	0	2
Not tested	2	6	2	3

Q1.2. GM maize identification in T1	Number of Laboratories				
	Present by screening	Present by event-specific PCR	Absent by screening	Absent by event-specific PCR	Not tested
No GM maize events tested	1	1	2	1	43
Maize 1507	1	1	4	9	12
Maize 3272	1	1	4	7	14
Maize 40278	1	1	3	6	17
Maize 5307	0	0	3	2	21
Maize 59122	1	1	4	9	12
Maize Bt11	1	1	5	9	11
Maize GA21	1	1	4	8	13
Maize MIR162	1	1	3	6	16
Maize MIR604	1	1	4	8	13
Maize MON810	1	1	5	10	11
Maize MON863	1	1	4	8	13
Maize MON87460	1	1	4	8	13
Maize MON88017	1	1	5	9	11
Maize MON89034	1	1	4	8	12
Maize NK603	1	1	4	9	12
Maize T25	1	1	5	8	12

Q1.3. GM soybean identification in T1	Number of Laboratories				
	Present by screening	Present by event-specific PCR	Absent by screening	Absent by event-specific PCR	Not tested
No soybean events tested	2	1	0	1	2
Soybean 305423	2	1	4	54	8
Soybean 356043	18	57	0	1	7
Soybean 40-3-2	1	1	31	33	6
Soybean 68416	1	2	17	37	12
Soybean A5547	3	0	25	38	7
Soybean A2704	3	1	26	36	6
Soybean CV127	2	0	3	45	20
Soybean FG72	0	1	24	24	20
Soybean MON87701	2	2	7	52	9
Soybean MON87705	1	1	26	27	15
Soybean MON87708	2	2	4	44	19
Soybean MON87769	3	1	6	38	22
Soybean MON89788	1	1	30	33	7

Q1.4. GM oilseed rape identification in T1	Number of Laboratories				
	Present by screening	Present by event-specific PCR	Absent by screening	Absent by event-specific PCR	Not tested
No GM OSR events tested	1	0	8	0	46
OSR 73496	1	0	3	2	18
OSR GT73	1	1	5	5	14
OSR MON88302	1	1	3	1	19
OSR MS8	0	0	5	5	15
OSR RF3	0	1	5	11	15
OSR T45	1	1	6	1	16

Q1.5. GM rice identification in T1	Number of Laboratories				
	Present by screening	Present by event-specific PCR	Absent by screening	Absent by event-specific PCR	Not tested
LLRice62	3	1	23	38	11

Q1.6. GM event identification in T2	Number of Laboratories				
	Present by screening	Present by event-specific PCR	Absent by screening	Absent by event-specific PCR	Not tested
Soybean 68416	12	51	0	1	10
Soybean A5547	2	1	14	51	4
Soybean MON87705	0	1	13	42	10

Q2. How was the DNA extracted from the test items?

Q2.1. Where did you get the DNA extraction method from?	T1	T2
a) ISO/CEN published method	24	21
b) EURL validated method	6	5
c) National reference method	4	2
d) International literature	4	4
e) In-house developed	4	6
f) Commercial kit	27	28

Q2.2. Which DNA extraction method or extraction kit did you use (Q2.3. Please specify)?	T1	T2
a) CTAB method	35	31
b) SDS method	3	3
c) Biotecon	2	2
d) GeneScan GeneSpin	4	4
e) Guanidine HCl with proteinase K	2	2
f) Macherey-Nagel NucleoSpin	9	11
g) Promega Wizard	3	3
h) Qiagen DNeasy plant mini kit	2	4
i) Qiagen DNeasy Mericon Food kit	1	0
j) Other	6	7

Q2.3. Please specify the DNA extraction method or kit, if not listed.	T1	T2
CTAB GenomicTip 20		1
Genetic ID Fast-ID Extraction kit	1	1
Qiagen DNeasy Blood & Tissue kit (modified)	2	2
Phenol-chloroform method	1	1
Generon Ion Force	1	1

Q2.4. Was the DNA further cleaned-up following use of the method specified above?	T1	T2
a) No additional DNA clean-up	41	42
b) Ethanol precipitation	5	4
c) Eurofins DNAExtractor Cleaning Columns	2	2
d) Promega Wizard DNA clean-up resin	5	5
e) Qiagen QIAQuick	4	3
f) Qiagen Genomic-Tip 20/G	0	1
g) Other	7	6

Q3. How many replicate DNA extractions were used to obtain the quanitative results(s) reported?	T1	T2
b) 2	47	48
c) 3	6	4
d) 4	7	7
f) 6	1	2
g) >6	2	1

Q4. Which general approach was used to analyse the test items?	T1	T2
a) Three-step analysis: screening - event identification - event quantification	48	24
b) Two-step analysis: screening - event quantification	2	3
c) Two-step analysis: event identification - event quantification	9	27
d) Two-step analysis: screening + event identification, then event quantification	2	1
e) One-step analysis: event quantification	0	1
f) No quantification was performed	4	7

Q5. Which real-time PCR instrument was used for quantification (not for qualitative analysis)?	No. of laboratories
b) ABI 7000	1
c) ABI 7300	4
d) ABI 7500	22
e) ABI 7700	1
f) ABI 7900 (HT)	14
g) ABI ViiA7	3
h) ABI StepOne & StepOne Plus real-time PCR system	1
i) BioRad iCycler	1
j) BioRad CFX	3
k) ABI QuantStudio	2
l) Corbett Rotor-Gene	2
m) Roche LightCycler 480	4
o) Roche LightCycler 2.0	1
p) Stratagene Mx	6

Q6. If applicable, which digital PCR instrument was used for quantification?	No. of laboratories
a) No digital PCR instrument was used	66

Q7. Which event-specific quantification methods were used (for both test items)?	No. of laboratories
a) Reference method from EURL GMFF GMOMETHODS database	60
d) ISO/CEN	2
e) In-house developed and optimised	1
f) International literature	1
h) No quantification was performed	4

Q8. Which endogenous target DNA sequence was used as taxon-specific reference gene for quantification?	T1	T2
No quantification performed	5	7
Maize hmg	8	1
Maize adh1-70 bp	2	1
Maize adh1-134 to 136 bp	4	0
Soybean lec	56	53
Oilseed rape CruA	9	10
Oilseed rape FatA(A)	1	1
Rice PLD	9	2
Rice GOS9	2	0
Other	2	1

Q9. How were the final quantitative results determined?

Q9.1. Which reference material was used for calibration?	T1	T2
CRM from IRMM, certified for GM mass fraction (g/kg)	54	47
CRM from IRMM, certified for GM DNA copy number ratio (plasmid calibrant)	1	0
CRM from AOCS, certified for GM presence (purity)	2	5
Non-certified RM (e.g. QC material), expressed in GM mass fraction	1	0
Non-certified RM, expressed in GM DNA copy number ratio (e.g. determined by digital PCR)	1	1
Not applicable	6	9

Q9.2. Test Item 1: Please specify the reference material used for calibration.	T1
ERM-BF425 series	50
ERM-AD425 pDNA	2
Plasmid standard pCR4-356043/Lec	1
ERM-BF410 series	1

Q9.3. Test Item 2: Please specify the reference material used for calibration.	T2
ERM-BF432 series	45
AOCS 0707	3
AOCS 0210	2
AOCS 0906	1

Q9.4. Was a conversion factor used to translate cp/cp% into m/m%?	T1	T2
No conversion necessary, all data are in m/m%	52	44
GM event is homozygous, cp/cp% is same as m/m%	4	5
No quantification was performed	7	10

Q9.5. Test Item 1: Please specify the actual conversion factor used, if applicable.	T1
1	1
2	2
Not applicable (NA)	60

Q9.6. Test Item 2: Please specify the actual conversion factor used, if applicable.	T2
1	2
2	2
Not applicable (NA)	58

Q10. How was the measurement uncertainty determined?	No. of laboratories
a) From the repeatability standard deviation of the test item measurements	38
b) From the within-laboratory reproducibility standard deviation (intermediate precision)	15
c) In another way	13
If c), please specify (Q18.1).	
U(expanded) = U(compound) * (k) IC 95%	1
Accredited methods: b) within-lab repro SD; Non accredited methods: a) Rep SD Test Item	1
Uncertainty=Coverage Factor (P=95% and f=n-1) * Standard Deviation / Square-root (Number	1
Relative standard deviation (ISO21570:2005)	1
From the EURL interlaboratory validation relative reproducibility standard deviation at 0.9% with k=2	2
Type B uncertainty	1
u = S/a √1/p + 1/n + (c0-c)2/Sxx	1
Formula used for the measurement uncertainty = 2*% RSD/2,83*2.5	1
RSD divided by root of replicates (8), multiplied by coverage factor (2.365; t-value for P(0.975; 7))	1
Both methods are not in house verified.	1
Not determined	2
Not applicable, no quantification	3

Q11. Additional comments

For T1 item we identified p35S by screening. Within the framework of this analyse and plant screening we just worked on the event that includes 35S or 35S/NOS (together) systems by event specific PCR. Except these events we marked the other events' title as "not tested". Some of these events on table are not in our analysing scope. All analyses were negative for T2 item and also soybean GM events.
The following methods are performed out of accreditation: Identification MON89769, Quantification DAS68416-4
We found this test is much more difficult for us.
The detection and quantification of soybean event 68416 in the test item T2 wasn´t performed because of the absence of consumables (oligonucleotides). It will be delivered at the end of May. For this reason we would like to ask you to send the results later.
We are used for Oilseed rape the taxon-specific reference gene PEP.
Only sample 1 was tested. A pre-spotted plate was used to screen the sample then 356043 soybean was quantified using 2x sample (extracted on different days) and 2x reference material (extracted on different days) to give a total of 4 quantifications which were averaged. Our laboratory deals primarily with mazie and oilseed rape so we did not have the reference material to quantify other events
According to respective contracts in North-Rhine-Westphalia the analysis of rice events was carried out in the Chemical and Veterinary Analytical Institute Rhein-Ruhr-Wupper.
We obtained positive result of 35s and Nos in sample T1, but around our limit of detection (~0,01%; method validated on feed and CRMs samples).
The DAS68416 event method has not yet been in house validated. DP356043 in sample 1 could not be quantified reliably.
Screening assays targeting P35S, Tnos, pat, cp4epsps were performed. Quantification of some of the events not conducted due to non availability of appropriate reference materials.
Test item 2 not quantified
Item 1 was tested for: P35S, TNOS, NPTII, PAT, CP4-EPSPS, CTP-EPSPS, 35S-HPT, CPTI-TNOS, CRYAC-TNOS, CRY1AB/AC-TNOS, CRY1AB/CRY1AC, P-UBI-CRY1AB, P35-HPT, P35S:BAR
T1: potato no detected; cotton no detected; sugar beet no detected; P35S detected; NPTII detected; T-nos no detected; CP4-EPSPS no detected; PAT no detected; CTP2-CP4EPSPS no detected; 35S/BAR no detected; BAR no detected
Nice questionnaire :-)
Item 1: was not investigated, Item 2: qualitative qPCR for A5547 and MON87705 was negative (<0.1 % m/m of total DNA, determined from comparison to 0.1% reference material), no screening procedure established for 68416
According to the screening elements results the sample T1 may be positive for Soybean 356043 (p35S positive). Unfortunately the Lab did not receive primers and probes for the particular modification on time so the event specific method was not performed.
The detection of soy events 305423, 68416, CV127 & MON87769 cannot be currently carried out in our laboratory due to delays of public procurement process of reagents. The results, obtained from the tests which have been performed, indicated that GM soybean DP 356043 had to be quantified. However, due to a limited availability of reagents, our lab could not submit reliable quantitative results.

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.

In general, the approach relies on the calculation of z-scores from $\log_{10}$-transformed data[17,18] based on the robust means[9,10] (μ_R) of the participants' results. The EURL GMFF calculated the consensus values from the participants' results taking the robust means (μ_R) for T1 and T2 on both original and $\log_{10}$-transformed scale, taking into account the agreed standard deviation ($\hat{\sigma}$) for comparative testing, set to 0.2 (T1) or 0.15 (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' results

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.

Table A4.1. Performance of "Category a" laboratories (NRL/882) in comparative test ILC-EURL-GMFF-CT-01/15 (- = not available, * = cannot be calculated).

Laboratory Code	Test Item 1			Test Item 2		
	356043 Soybean (μ_R = 1.34 m/m %)			68416 Soybean (μ_R = 0.46 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	Z-score	Result (m/m%)	Uncertainty (m/m %)	Z-score
L04	-	-	-	0.41	0.07	-0.29
L07	1.04	0.31	-0.49	0.40	0.14	-0.36
L12	1.51	0.45	0.32	0.40	0.12	-0.36
L14	1.36	0.40	0.09	0.61	0.05	0.86
L19	0.89	0.42	-0.83	0.45	0.13	-0.02
L22	2.31	0.69	1.24	0.39	0.18	-0.43
L26	1.20	35.03	-0.18	0.64	8.07	1.00
L27	1.02	0.33	-0.53	0.41	0.14	-0.29
L28	2.90	0.30	1.73	0.35	0.06	-0.75
L29	1.19	0.30	-0.20	0.50	0.16	0.28
L30	0.68	0.16	-1.42	0.23	0.12	-1.96
L31	0.94	0.62	-0.71	0.49	0.16	0.23
L40	1.21	-	-0.16	0.39	-	-0.43
L42	<0.1	-	*	0.50	-	0.28
L43	1.25	0.22	-0.09	0.49	0.17	0.23
L44	-	-	-	0.46	0.08	0.04
L47	1.92	0.54	0.84	-	-	-
L49	1.46	0.53	0.24	0.32	0.12	-1.01
L50	1.40	0.20	0.15	0.50	0.10	0.28
L51	0.96	0.29	-0.67	0.47	0.14	0.11
L53	1.08	0.27	-0.41	-	-	-
L54	1.15	0.10	-0.27	0.45	0.06	-0.02
L55	1.24	0.33	-0.11	0.52	0.07	0.39
L59	1.63	0.34	0.48	0.46	0.08	0.04
L64	1.00	-	-0.58	0.60	-	0.81
L68	1.24	-	-0.11	0.49	-	0.23
L71	-	-	-	0.52	0.16	0.40

Table A4.2. Performance of "Category b" laboratories (NRL/120) in comparative test ILC-EURL-GMFF-CT-01/15 (- = not available).

Laboratory Code	Test Item 1			Test Item 2		
	356043 Soybean (μ_R = 1.34 m/m %)			68416 Soybean (μ_R = 0.46 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	Z-score	Result (m/m%)	Uncertainty (m/m %)	Z-score
L06	1.39	0.18	0.14	0.43	0.09	-0.15
L10	1.70	0.39	0.57	0.36	0.11	-0.67
L13	1.55	0.20	0.37	0.48	0.04	0.17
L18	2.64	0.41	1.53	0.27	0.04	-1.50
L20	1.70	0.30	0.57	0.49	0.30	0.23
L33	1.35	0.10	0.07	0.34	0.14	-0.83
L35	0.94	0.22	-0.71	0.49	0.06	0.23
L36	0.17	0.08	-4.48	0.74	0.10	1.44
L37	2.46	-	1.38	-	-	-
L46	1.00	0.30	-0.58	0.46	0.14	0.04
L52	1.81	0.19	0.71	0.61	0.05	0.86
L56	1.52	0.61	0.33	0.46	0.07	0.04
L62	1.52	0.22	0.33	<0.1	-	*
L65	1.56	0.22	0.39	0.35	0.02	-0.75
L66	-	-	-	0.26	0.04	-1.61
L67	1.30	-	-0.01	0.40	-	-0.36
L69	1.20	0.49	-0.18	0.42	0.17	-0.22

Table A4.3. Performance of "Category c" laboratories (non-NRL) in comparative test ILC-EURL-GMFF-CT-01/15 (- = not available, * = cannot be calculated).

Laboratory Code	Test Item 1			Test Item 2		
	356043 Soybean (μ_R = 1.34 m/m %)			68416 Soybean (μ_R = 0.46 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	Z-score	Result (m/m%)	Uncertainty (m/m %)	Z-score
L01	>0.1	-	*	-	-	-
L03	0.89	0.18	-0.83	0.52	0.10	0.40
L08	1.40	-	0.15		-	-
L09	0.28	-	-3.34	0.38	-	-0.55
L21	0.95	0.33	-0.69	-	-	-
L24	1.75	0.50	0.64	-	-	-
L32	1.25	-	-0.09	-	-	-
L34	0.69	0.20	-1.38	0.88	0.26	1.92
L38	1.20	0.60	-0.18	0.50	0.30	0.28
L39	1.30	0.32	-0.01	0.77	0.16	1.53
L58	1.95	0.42	0.87	0.44	0.08	-0.09
L61	1.76	0.38	0.65	0.43	0.08	-0.15
L70	-	-	-	0.52	-	0.37
L72	1.81	-	0.71	0.44	-	-0.09

Figure A4.1. Z-scores for soybean event 356043 in Test Item 1 on the basis of a robust mean of 1.34 m/m % (◊).
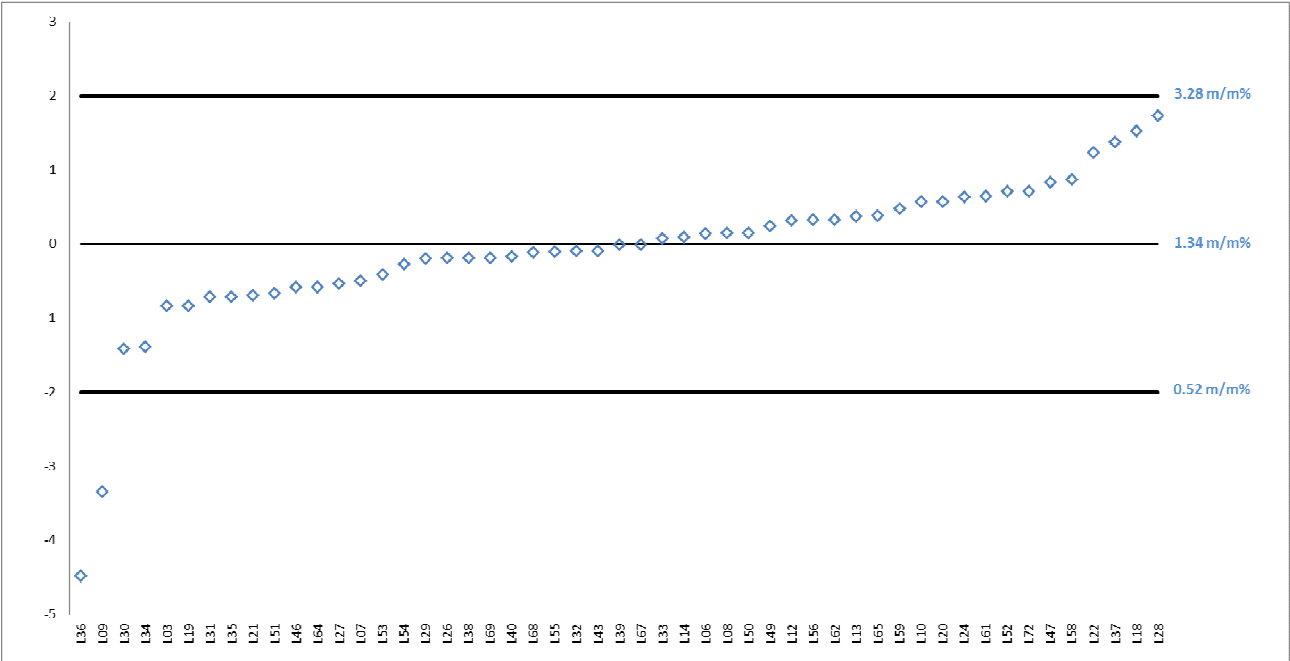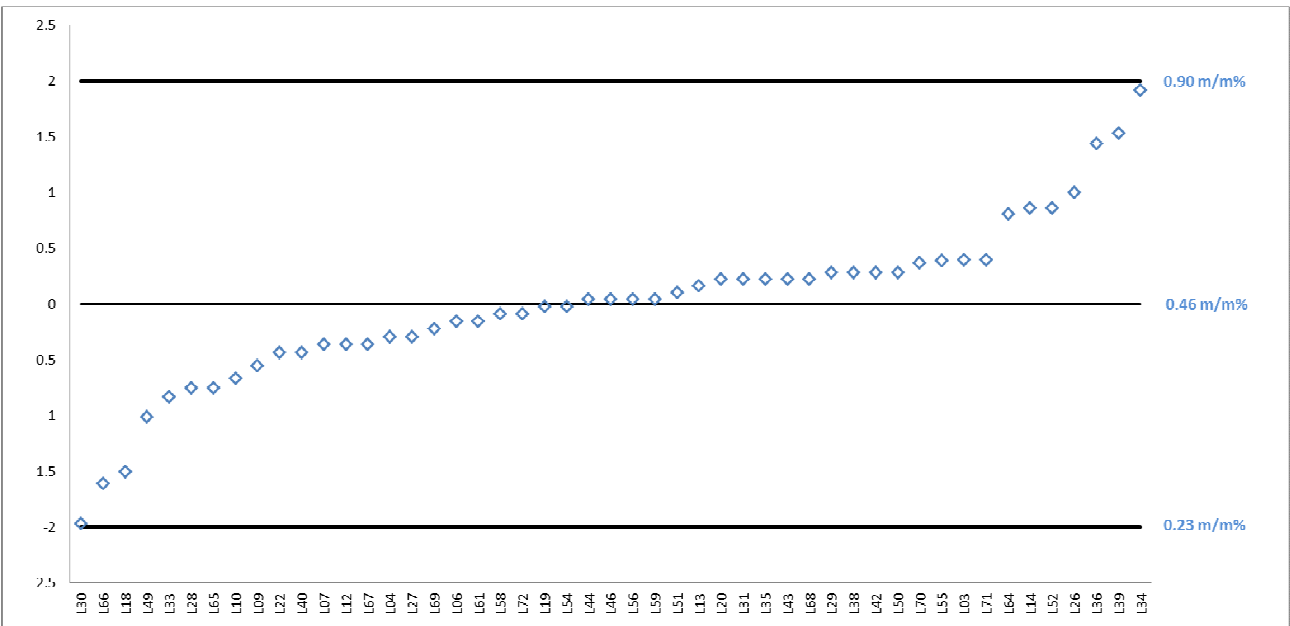

Figure A4.2. Z-scores for soybean event 68416 in Test Item 2 on the basis of a robust mean of 0.46 m/m % (◊).

Annex 5: Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 19 March 2015
JRC.DG.I.3/MBG/JK/wb/lv

NOTE FOR THE ATTENTION OF

I. **All National Reference Laboratories designated under COMMISSION REGULATION (EC) No 882/2004**

II. **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 120/2014**

III. **All members of the European Network of GMO Laboratories**

IV. **Official control laboratories**

V. **Interested parties from third countries**

Subject: Invitation to participate in the comparative test ILC-EURL-GMFF-CT-01/15

Dear Colleague,

Hereby, I would like to invite you to participate in the 11th round of comparative testing ILC-EURL-GMFF-CT-01/15, organised by the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) in line with its mandate under Regulation (EC) No 882/2004. This round of comparative testing will include two different test materials and the following tasks are requested from the participants:

Test Item 1: "Food"
- Perform species identification (maize, soybean, oilseed rape and rice);
- Identify and quantify the GM event(s) detected.

Test Item 2: "Soybean Flour"
- Screen for the presence of the following three GM soybean events: 68416, A5547, MON 87705;
- Quantify the GM event(s) detected.

Your participation is free of charge. As communicated previously, the quantitative results have to be reported in mass/mass %. Results reported in copy/copy % will not be evaluated. You are requested to provide further details on your analysis in a questionnaire that is part of the information to be reported to the EURL GMFF. If a conversion factor was applied to convert measurement data in cp/cp % into data in m/m % this needs to be reported in the questionnaire.

ISO 9001:2008 certified by

Joint Research Centre · I-21027 Ispra (VA), Italy · TP 331
Telephone: direct line (+39)0332/786735, · Telefax: (+39)0332/786159
E-mail: Joachim.kreysa@ec.europa.eu
http://ihcp.jrc.ec.europa.eu

CERMET
SISTEMA
DI GESTIONE
CERTIFICATO
SINCERT

I would like to remind you that participation in comparative testing is mandatory for all National Reference Laboratories designated under Regulation (EC) No 882/2004. The participation of National Reference Laboratories nominated under Regulation (EU) No 120/2014 is not mandatory though highly recommended. 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 in line with Regulation (EC) No 882/2004 to DG SANTE for the purpose of an assessment of their performance.

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

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1401

Please be aware that each laboratory can only register once for this comparative testing round. You are requested to return the signed registration form to us by E-mail (not by fax!).

The deadline for registration is **Monday 30 March 2015**. Samples will be shipped during the week of **6 April 2015** (week after Easter). The deadline for submission of the results is **Thursday 21 May 2015**. Please be aware that results submissions after the deadline will not be accepted.

Please contact the functional mailbox mbg-comparative-testing@jrc.ec.europa.eu for all issues related to this comparative testing round, *e.g.* difficulties with your on-line registration, communications and questions related to the content of the comparative testing round.

The EURL GMFF is looking forward to your participation.

Yours sincerely,

Joachim Kreysa
Head of Molecular Biology and Genomics Unit
Joint Research Centre of the European Commission

Annex 6: Accompanying letter to shipment of samples

Ref. Ares(2015)1459840 - 02/04/2015

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

EURL
European Union Reference Laboratory
for GM Food & Feed

JRC.DG.I.3/MBG/JK/wb/lv/

NOTE FOR THE ATTENTION OF

All Laboratories registered for the comparative test ILC-EURL-GMFF-CT-01/15

«Firstname» «Surname» «LCode»
«Organisation»
«Address»
«Zip» «Town»
«Country»

Subject: **ILC-EURL-GMFF-CT-01/15, a comparative testing round to determine the GM content in two test materials, *i.e.* a food and a soybean flour.**

Dear Dr «Surname»,

Thank you for participating in the ILC-EURL-GMFF-CT-01/15 comparative testing round. Please find in this parcel two test materials, each composed of a different matrix.

The parcel contains:
1. Two plastic containers each containing approximately 5 g of test item;
2. An "Acknowledgement of Reception" form.

Please check whether the plastic containers containing the test item remained undamaged during transport and return the signed "Acknowledgement of Reception" form by e-mail with scanned pdf (preferred), or by fax (+39 0332 786159). You should store the samples in a dark and cold place (not exceeding 4 °C).

Tasks
Participants should perform the following tasks:

Test Item 1: "Food"
- Perform species identification;
- Screen for the presence of GM events;
- Quantify the GM event(s) detected.
Test Item 2: "Soybean Flour"
- Screen for the presence of the following three GM soybean events: 68416, A5547, MON 87705;
- Quantify the GM event(s) detected.

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

The quantitative results have to be reported in mass/mass % (not accepted: copy/copy %), as outlined below:

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

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2

Please be aware of the following rules:

- 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 %, without any need for conversion to cp/cp %, while 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) need to be converted into m/m %, using a conversion factor of your choice (to be detailed in the questionnaire); further guidance has been published by the EURL GMFF[1];
- Results reported through the reporting website can only be expressed in m/m %.

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

After entering all results, please complete the questionnaire on-line (you will also receive a pdf file of this questionnaire in an E-mail to be used as an aid in the laboratory). In the questionnaire, items bearing an info icon "i" on the right-hand side contain additional information for the participant. In the reporting website clicking on the icon will give access to this information. Do not forget to save, submit and confirm when required to do so.

Only results and answers to the questionnaire that are reported <u>on-line</u> on the reporting website https://web.jrc.ec.europa.eu/ilcReportingWeb will be accepted.

Directly after submitting your results and the questionnaire information on-line, you will be prompted to print the completed report form. Please sign the printed report form and return it to the EURL GMFF as scanned pdf by E-mail (mbg-comparative-testing@jrc.ec.europa.eu). Check your results carefully before submission, since this is your final confirmation. The EURL GMFF will not verify your data.

The deadline for submission of results is <u>Thursday 21 May 2015</u>. It will not be possible to submit your results after the deadline.

Please contact the functional mailbox mbg-comparative-testing@jrc.ec.europa.eu for all issues related to this comparative testing round.

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

Yours sincerely,

P.O.

Joachim Kreysa
Head of Molecular Biology and Genomics Unit
Joint Research Centre of the European Commission

Annex 7: Confirmation of shipment

Our Ref: Ares(2015)1506498

Dear Participant,

Your test parcels related to the 11th comparative testing round ILC-EURL-GMFF-CT-01/15 left our premises yesterday, 7 April 2015, by TNT courier.
For your convenience, please find herewith the corresponding tracking number you could refer to in order to track the relevant materials on the Web:

«Tracking_number»

The parcel with test items that you will receive should contain:

- One plastic container with two samples, each containing approximately 5 g of test item;
- An "acknowledgement of reception" form, that should be returned to the **EU-RL GMFF** as scanned pdf by e-mail to mbg-comparative-testing@jrc.ec.europa.eu;
- An accompanying letter**.**

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.

Your Lab Code (Lxx) is indicated in the accompanying letter as well as in the "acknowledgement of reception" form in the upper right side of the page; please keep it for future uses in this CT round.

The deadline for submission of your results is **21 May 2015.**

The questionnaire (which will need to be filled in online on the reporting website) will be sent via separate e-mail.

Please contact only the functional mailbox mbg-comparative-testing@jrc.ec.europa.eu for any issue related to this comparative testing round.

Thank you for your collaboration.

Lorella Vidmar
On behalf of

The Comparative Testing staff

European Commission
DG Joint Research Centre

Institute for Health and Consumer Protection
Unit I.3 Molecular Biology and Genomics
TP 201 Via E. Fermi 2749
I-21027- Ispra (VA) Italy

Functional mailbox: mbg-comparative-testing@jrc.ec.europa.eu

Annex 8: Acknowledgement of receipt

![DG JRC I3]	**FAX - Record for Quality System**	**EURL** European Union Reference Laboratory for GM Food & Feed
JRC.I3.R71/EURL Date: 19/07/2011 Revision. 4	**Acknowledgement of reception**	Page 1/1

From :

Lab Code:

To : Molecular Biology and Genomics Unit
    Method Validation / EURL-GMFF
    European Commission - Joint Research Centre - IHCP
    21027   ISPRA (VA) Italy

fax: +39 0 332 78 6159

File nb EURL-CT-01/15

In good condition

We have received the following samples   Yes ☐   No ☐

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 EMAIL to:
mbg-comparative-testing@jrc.ec.europa.eu

References

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Stimulating innovation
Supporting legislation