European Commission

JRC TECHNICAL REPORT

Comparative Testing Report on the Detection and Quantification of GM Events in Instant Soup and Soybean Flour

Comparative testing round: ILC-EURL-GMFF-CT-02/15

European Union Reference Laboratory for Genetically Modified Food and Feed

2015

Joint Research Centre

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

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Abstract

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 instant soup spiked with oilseed rape GM event MON88302 (Test Item 1, T1) and a sample composed of soybean flour containing soybean event 81419 (Test Item 2, T2). Participants were requested to identify which plant species and GM events were present in T1, and to identify which of any of three given GM soybean events were present in T2. Any GM event detected in T1 and T2 should have been quantified and the results reported in GM mass/mass %.
Seventy-four participants from 36 countries participated to this CT round. Seventy-one laboratories (96 %) correctly reported the presence of oilseed rape in T1, and all 56 laboratories which tested for the oilseed rape event MON88302 identified it. For T2, all 60 laboratories which tested for soybean event 81419 also identified it. Approximately 20 % of laboratories did not test for the specific GM event present in T1 and T2.
Fifty-seven laboratories returned quantitative results for one or both GM events based on event-specific quantitative real-time PCR. The EURL GMFF calculated the robust mean (μR) of the participant's results for oilseed rape event MON88302 in T1 (N = 44), used as the assigned value. T2 comprised re-labelled bottles of the certified reference material (ERM-BF437d) for soybean event 81419 and therefore the certified value was used as the assigned value. Z-scores were determined for the participants' results, based on these assigned values and the target standard deviations agreed by the Advisory Board for Comparative Testing. Quantification of oilseed rape event MON88302 in T1 resulted in a satisfactory performance ($|z| \leq 2.0$) for all but three laboratories (93 %). For soybean event 81419 in T2, all but two laboratories which had provided a quantitative result obtained a satisfactory z-score (96 %). Follow-up actions will be organised for the five laboratories which received an unsatisfactory z-score in this CT round.
Notably, of the 74 participants in this CT round, 30 and 23 participants for T1 and T2, respectively, did not report a quantitative result for the GM event present. Therefore, their performance for the quantification of these events could not be evaluated.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

EURL
European Union Reference Laboratory
for GM Food & Feed

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Executive Summary

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Seventy-four participants from 36 countries participated to this CT round. Seventy-one laboratories (96 %) correctly reported the presence of oilseed rape in T1, and all 56 laboratories which tested for the oilseed rape event MON88302 identified it. For T2, all 60 laboratories which tested for soybean event 81419 also identified it. Approximately 20 % of laboratories did not test for the specific GM event present in T1 and T2.

Fifty-seven laboratories returned quantitative results for one or both GM events based on event-specific quantitative real-time PCR. The EURL GMFF calculated the robust mean (μ_R) of the participant's results for oilseed rape event MON88302 in T1 ($N = 44$), used as the assigned value. T2 comprised re-labelled bottles of the certified reference material (ERM-BF437d) for soybean event 81419 and therefore the certified value was used as the assigned value. Z-scores were determined for the participants' results, based on these assigned values and the target standard deviations agreed by the Advisory Board for Comparative Testing. Quantification of oilseed rape event MON88302 in T1 resulted in a satisfactory performance ($|z| \leq 2.0$) for all but three laboratories (93 %). For soybean event 81419 in T2, all but two laboratories which had provided a quantitative result obtained a satisfactory z-score (96 %). Follow-up actions will be organised for the five laboratories which received an unsatisfactory z-score in this CT round.

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Content

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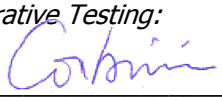
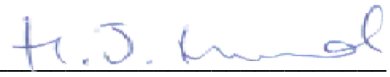
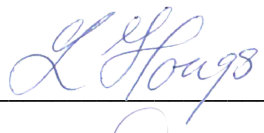
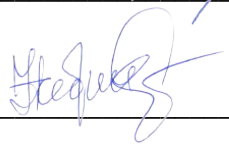
P. Corbisier 

H. Hird 

L. Hougs 

N. Papazova 

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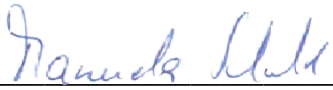
M. Schulze 

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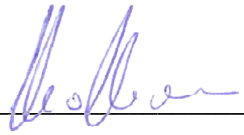
M. Mazzara (Competence group leader) 

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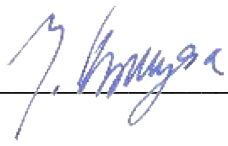
J. Kreysa (Head of unit) 

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 co-ordinate 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 of food and feed, thereby ensuring the quality and uniformity of analytical results obtained on routine test samples.

This report summarises the results obtained in the 12th CT round organised by the EURL GMFF since 2010. Participation in this CT round was 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 managed the IT tool for online registration and results submission and was responsible for data evaluation and laboratory performance assessment. This activity was supported by an Advisory Board for CT.

2. Test items

Two test items were prepared by the EURL GMFF: Test Item 1 (T1) consisted of instant soup, spiked with GM oilseed rape event MON88302 (unique identifier MON-883Ø2-9); T2 comprised re-labelled bottles of the certified reference material (ERM-BF437d) for soybean event 81419 (unique identifier DAS-81419-2), certified to contain 9.9 g/kg soybean event 81419[6].

T1 was prepared from powdered instant soup ("*Zuppa di verdure*") bought at a local market. Preliminary analysis indicated that DNA of a quality and quantity suitable for PCR analysis was extracted from this powder. Analysis of the DNA with event-specific pre-spotted plates[7] identified the presence of oilseed rape and very low levels of soybean and maize (Ct around 40). No GM events were detected. The instant soup was ground by the EURL GMFF using an Ultra Centrifugal Mill ZM200 (Retsch GmbH, DE). To increase the oilseed rape content in the material, non-GM oilseed rape was added to obtain a total oilseed rape concentration in the mixture of approximately 4.5 m/m %. GM oilseed rape event MON88302 (from the CRM AOCS 1011-A[8]) was then spiked in the dry powder to an approximate target concentration of 0.9 m/m % relative to the approximate oilseed rape content.

The non-GM oilseed rape, corresponding to CRM AOCS 0304-A[9], and the MON88302 oilseed rape (AOCS 1011-A), both consisted of ground flours which were considered to be sufficiently fine for inclusion into the test material without any additional grinding. An oven-drying method was used to determine the remaining water content in the base materials used for preparing T1 (Table 1). 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 that DNA could be extracted from all base materials with both methods, however, the CTAB method was less efficient than the NucleoSpin method for extraction from the oilseed rape flours, even after taking into account the lower sample intake for the CTAB method.

The quality and purity of the extracted DNA was tested as follows. Four NucleoSpin DNA extracts were randomly chosen from the 10 replicates for each T1 base material and were assessed for the presence of inhibitors. Inhibition tests on the DNA from the instant soup, non-GM oilseed rape and MON88302 oilseed rape were performed using the validated *ccf* reference gene system QT-TAX-BN-002 (http://gmo-crl.jrc.ec.europa.eu/gmomethods), using 200 ng DNA in 50 µL, in line with the validated quantitative PCR (qPCR) method for MON88302 oilseed rape EURL-VL-09-11-VM-MON88302. No inhibition was detected. Furthermore, the DNA extracts (100 ng in 50 µL) were assessed for the presence of GM events and species-specific DNA other than those relevant to this CT round, using event-specific pre-spotted plates[7]. In the DNA from AOCS 0304-A oilseed rape, GT73 was detected at very low levels (Ct around 40). In the AOCS 1011-A DNA (MON88302), GT73 was detected (Ct around 38), as well as traces of RF3, MS8 and the cotton event 3006 (Ct >40).

The final test item T1 was gravimetrically prepared in accordance with ISO Guide 34[10] ('General Requirements for the Competence of Reference Material Producers'), as follows:

- The nominal mass fraction of the GM material was produced by mixing the three 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	Instant vegetable soup	6.1	1379.3
	Conventional oilseed rape (AOCS 0304-A)	4.40	50.05
	MON88302 oilseed rape (AOCS 1011-A)	3.910	0.637
Test Item 2	81419 soybean (ERM-BF437d)	-[a]	-[a]

[a] Test Item 2 was prepared and characterised by IRMM as part of the certification of ERM-BF437d.

From the T1 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: "Instant soup"; T2: "Feed, Soybean") and stored at 4 °C. Assessment of the T1 flour with screening pre-spotted plates revealed the presence of oilseed rape and potato, the presence of traces (Ct > 40) of soybean and maize, and the absence of sugarbeet, rice and cotton; the CTP2-EPSPS genetic element, present in MON88302, was detected, as expected, but no other genetic elements commonly found in GM events were found.

Homogeneity and stability testing of T1 was carried out in-house. 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 T1 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.

Homogeneity and stability of T2 were confirmed by IRMM as part of the certification procedure and were not repeated.

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: "Instant soup":

- Perform species identification (maize, soybean, oilseed rape and rice);
- Identify and quantify the GM event(s) detected;

For Test Item 2:

- Screen for the presence of the following three soybean GM events:
  - MON89788, 68416 and 81419;
- Quantify the event(s) detected.

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

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

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 a conversion factor of their own (to be detailed in the questionnaire); further guidance has been published by the EURL GMFF[11].

4. Results

4.1 Participation to CT round 02/15

In May 2015, a total of 189 laboratories were invited to participate in the CT round ILC-EURL-GMFF-CT-02/15 and 83 laboratories registered for it. Three laboratories cancelled their participation before or during shipment and six others did not submit their analysis data. Seventy-four laboratories from 36 countries returned results within the reporting deadline. 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-two NRLs designated under Regulation (EC) No 882/2004 (NRL/882);
b) Eighteen NRLs nominated only under Regulation (EU) No 120/2014 (NRL/120);
c) Twenty-four official control laboratories, but not NRLs nominated under either Regulation. This category included 10 EU laboratories and 14 laboratories from non-EU countries.

Table 2. Participation in the comparative testing round ILC-EURL-GMFF-CT-02/15.

Date of invitation[a]	26 May 2015
Date of shipment of samples[a]	16+17 June 2015
Deadline results submission[a]	21 August 2015
Number of invited laboratories	189
Number of registered laboratories	83
Registered laboratories that failed to submit their data	L04, L16, L50, L52, L62, L70, L75, L81, L83
Number of participating laboratories	74
Laboratories submitting only qualitative data (GM identification)	L02, L03, L12, L21, L22, L23, L26, L34, L39, L48, L57, L58, L61, L64, L68, L73, L74
Number of laboratories with quantitative data (GM quantification)	57

[a] The official letters used for communication with the (potential) participants are shown in the Annexes.

Table 3. Overview of participants by country and category.

Country	Number of participants	NRL/882 Cat. (a)	NRL/120 Cat. (b)	Non-NRL Cat. (c)
EU				
AUSTRIA	2	2		
BELGIUM	3	3		
BULGARIA	2	1		1
CROATIA	2	1		1
CYPRUS	1	1		
CZECH REPUBLIC	1	1		
DENMARK	1	1		
ESTONIA	(a)	(a)		
FINLAND	2	1	1	
FRANCE	1	1		
GERMANY	17	1	13	3
GREECE	1	1		
HUNGARY	2	1		1
IRELAND	(a)	(a)		
ITALY	3	1	1	1
LATVIA	1	1		
LITHUANIA	1	1		
LUXEMBOURG	1	1		
MALTA	(a)	(a)		
NETHERLANDS	2	1	1	
POLAND	4	3		1
PORTUGAL	1	1		
ROMANIA	2	1		1
SLOVAKIA	2	2		
SLOVENIA	1	1		
SPAIN	2	2		
SWEDEN	1	1		
UNITED KINGDOM	4	1	2	1
Total EU	60	32	18	10
Non-EU				
ARGENTINA	1			1
CHILE	1			1
COLOMBIA	1			1
HONG KONG	1			1
INDIA	1			1
MEXICO	1			1
SERBIA	2			2
SWITZERLAND	2			2
TURKEY	2			2
UKRAINE	1			1
VIETNAM	1			1
Total non-EU	14			14
Total	**74**	**32**	**18**	**24**

(a) This country delegates its NRL activities to a participating laboratory from the UK.

4.2 Information on the testing provided in the questionnaire

Participants were asked to fill in a questionnaire on their testing approach for T1 and T2, consisting of 9 main questions, and several sub-questions, which were mostly in a multiple-choice format. Table 4 summarises the main answers received; Annex 2 shows all answers.

Table 4. Summary of information provided in the questionnaire of CT 02/15.

Subject of Question	Question Number	Test Item	Main Answers[1]
Test for PCR inhibition	Q1.7	T1	Compare CT and/or GM % of two dilutions (62 %) Not done (20 %)
		T2	Compare CT and/or GM % of two dilutions (61 %) Not done (16 %)
DNA extraction method	Q2	T1	CTAB (39 %) or commercial kit (61 %), mainly NucleoSpin (24 %) No DNA clean-up (66 %), or ethanol precipitation (11 %)
		T2	CTAB (38 %), or commercial kit (62 %), mainly NucleoSpin (20 %) No DNA clean-up (68 %), or ethanol precipitation (11 %)
Number of DNA extracts analysed	Q3	T1	2 extracts (84 %) 4 extracts (18 %)
		T2	2 extracts (75 %) 4 extracts (20 %)
General approach of analysis	Q4	T1	Three-step (Screening-Identification-Quantification; 89 %) Two-step (Screening+Identification-Quantification; 11 %)
		T2	Two-step (Identification-Quantification; 57 %) Three-step (Screening-Identification-Quantification; 35 %)
Real-time PCR instrument used	Q5	T1 & T2	ABI (58 %, mainly 7500 & 7900)
Event-specific method used	Q6	T1	EURL GMOMETHODS database (91 %)
		T2	EURL GMOMETHODS database (94 %)
Endogenous target DNA sequences used	Q7	T1	Oilseed rape CruA or Ccf (73 %)
		T2	Soybean lectin-74 bp (82 %)
Reference material used	Q8	T1	CRM from AOCS (95 %), AOCS 1011-A Data expressed in m/m% without conversion (91 %)
		T2	CRM from IRMM (98 %), ERM-BF437 Data expressed in m/m% without conversion (100 %)
Measurement uncertainty approach	Q9	T1	Calculated from repeatability (64 %), from reproducibility (27 %)
		T2	Calculated from repeatability (57 %), from reproducibility (25 %)

[1] For Q1.7 and Q2, the percentages shown are per total number of participants (74); for the other questions, percentages are expressed per number of participants that provided a quantitative result for the correct event in T1 (44) or T2 (51).

In general, laboratories prepared two DNA extracts per test item using either a commercial kit or a CTAB method, both without additional clean-up. The DNA quality in the extracts (inhibition) was tested by comparing the results of two dilutions of the extracts. Quantitative analysis was performed using the EURL-validated real-time PCR methods from the GMOMETHODS database. Oilseed rape *CruA* or *ccf* (both cruciferin targets) were used as endogenous taxon-specific reference gene for T1 (as *ccf* was not listed in the multiple choice tables, participants may have ticked the *CruA* box when *ccf* was actually used). For soybean, the lectin *Le*1-74 bp target was used by most participants. The CRMs from AOCS and IRMM were used for calibration of the measurements for MON88302 and 81419, respectively, and the results were expressed in m/m %, without the need for use of a factor to convert results expressed in copies to mass. 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, but most participants (57 %) followed a two-step approach in this case, i.e. event-specific identification, then quantification.

4.3 Species identification

Nearly all laboratories (71 out of 74) reported the presence of oilseed rape in T1 (93 %), and most reported the absence of the other crop species (Table 5). One laboratory (L26) reported that oilseed rape was absent, and only soybean present, but no GM events were identified nor quantified by this laboratory.

Species identification was not requested for T2.

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	18	71	10	6
Absent	56	1	63	62
Not tested	0	2	1	6

4.4 GM event identification

The questionnaire included tables for reporting the presence or absence of the GM events tested in each test item, and the analytical approach used (by GM screening and/or event-specific 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). As T1 was labelled as a food matrix (instant soup), Regulation (EU) 619/2011 does not apply and any trace of these GM events would be considered unauthorised. When a participant had determined the absence of the species in the first screening tests (Section 4.3), they could tick the "No GM [species] events tested" button (species referring to maize, soybean, or oilseed rape), without the need 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 for GM event identification. In both test items the correct GM events were identified by the majority of the 74 participants, based on event-specific qualitative analysis or screening. All laboratories (100 %) which had tested for oilseed rape MON88302 and 81419 soybean in T1 (N = 56) and T2 (N = 59) respectively, reported the presence of that event. However, 18 (24 %, T1) and 14 (19 %, T2) laboratories did not test for these events.

A few additional GM events were quantified, mostly reported by one participant (L34). This participant had also falsely reported the presence of many events in one test item in a previous CT round.

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
	Oilseed Rape MON88302	Soybean 81419
Present by screening	30	7
Present by event-specific PCR	50	56
Absent by screening	0	0
Absent by event-specific PCR	0	1[a]
Not tested	18	14

[a] This was probably a mistake, as the laboratory (L18) has provided a quantitative result for this event.

4.5 GM event quantification

4.5.1 Quantitative results reported by the participants

Of the 74 laboratories that participated to this CT round, 57 participants submitted event-specific quantitative data for one or both GM events (Table 2). A number of laboratories only quantified either MON88302 in T1 or 81419 in T2, and two laboratories (L39 and L54) reported semi-quantitative values for MON88302 (above 0.01 and 0.05 m/m %, respectively). A total of 44 quantitative values were obtained for event MON88302 in T1 and 51 for event 81419 in T2. No quantitative data were reported for any other GM events in T1 or T2. Among the 32 NRL/882 participants (category a) in this CT, only 22 provided quantitative data for oilseed rape MON88302 in T1, and 27 for soybean 81419 in T2; two NRL/882 participants (L21 and L74) provided no quantitative data at all.

Measurement uncertainties were reported for 86 % of all reported measurement results, and a coverage factor was reported for 78 % of the results. One laboratory (L14) returned a relative measurement uncertainty for soybean 81419 (in % of the quantitative value).

Two participants (L01 and L55) had reported the use of a conversion factor (x 2) for MON88302, but explained later that they had misunderstood the text in the validation report. The correct use of the CRM for MON88302 from AOCS, which is certified for MON88302 purity and consists of ground MON88302 oilseed rape, is to consider this CRM as 100 % in mass fractions of GM DNA (100 m/m %) MON88302, but 50 cp/cp % (as the *ccf* reference gene is present in two gene copies on the haploid genome). Two approaches can be used to prepare the calibration standards (at 40 ng/µL):

1. The standards are expressed in m/m %: the DNA extracted from the CRM is diluted 10 times in non-GM oilseed rape DNA (e.g. extracted from AOCS 0304-A) to prepare the first standard S1 (10 m/m % GM, 100 m/m % *ccf*), then further dilutions are made in water or TE to prepare the GM standard curve (down from 10 %) and the reference gene standard curve (down from 100 %). The final measurement results (GM %) will be expressed in m/m % without any need for a conversion factor;

2. The standards and all measurement data are expressed in cp/cp %: the DNA extracted from the CRM is diluted 5 times in non-GM oilseed rape DNA (e.g. extracted from AOCS 0304-A) to prepare the first standard S1, which corresponds approximately to 10 cp/cp % (~ 34.783 copies of MON88302 and ~ 347.826 copies of *ccf*), then further dilutions are made in water or TE, using the approximate copy numbers of GM and reference gene for both standard curves. The measurement results will be expressed in cp/cp % and need to be converted into m/m % by multiplication by a

factor two (for this oilseed rape event and assuming the zygosity of the unknown sample is equal to that of the CRM used).

4.5.2 Assigned values

The assigned value for MON88302 in T1 was based on the consensus value (μ_R) for the data from participants in the CT round, calculated using robust statistics[12,13]. This approach minimises the influence of outlying values.

The expanded uncertainty (U) on the result obtained comprises standard uncertainty (u) contributions from the characterisation of the material (u_{char}) and the between-test item homogeneity (u_{bb})[14], and is estimated for MON88302 according to:

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

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:

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

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

For 81419 soybean, the certified value of ERM-BF437d was used as the assigned value and the expanded uncertainty was taken from the certificate of this CRM. For information, the robust mean calculated from the participants' results reported for this event was 0.95 m/m %, which was close to the assigned (certified) value.

The assigned values and associated uncertainties for both GM events are reported in Table 7.

Table 7. Overview of assigned values and expanded uncertainties for oilseed rape event MON88302 and soybean event 81419 in test items T1 and T2, respectively.

Test Item	GM Event	Approach used	Assigned value (m/m %)	Expanded uncertainty (m/m %)
T1	MON88302 Oilseed Rape	Robust Mean (N = 44)	1.16	0.18
T2	81419 Soybean	Certified Value	0.99	0.15

4.5.3 Performance of the laboratories providing quantitative results

To evaluate laboratory performance, z-scores were calculated for both GM events on the basis of the assigned value for each event (see Annex 3, formulas A3.1-A3.2). Based on the experience in previous CT rounds and taking into account the results of previous CTs, 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. 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.

Three laboratories, including one NRL/882, received a z-score outside the acceptable range (i.e. |z| > 2.0) for oilseed rape event MON88302 in T1. Whilst all other results were within the range of 0.44 – 2.76 m/m %, these three laboratories reported the MON88302 content as 0.16 (L15), 0.29 (L10) and 0.34 m/m % (L05).

All but two laboratories (L25 and L79) performed satisfactorily for the quantification of soybean event 81419 in T2. These two laboratories had reported values widely outside the acceptable range of 0.50 – 1.98 m/m %. All NRL/882 laboratories as well as all but five other laboratories performed very well for this event, with absolute z-scores ≤ 1.0.

Table 8. Performance of laboratories in comparative test ILC-EURL-GMFF-CT-02/15 for quantification of oilseed rape event MON88302 (T1) and soybean event 81419 (T2).

Test Item	GM Event	Satisfactory z-score	Unsatisfactory z-score
T1	MON88302 Oilseed Rape	L01, L06, L07, L08, L09, L13, L17, L19, L20, L24, L27, L28, L29, L30, L31, L32, L33, L35, L36, L37, L38, L40, L41, L42, L45, L46, L47, L49, L53, L55, L56, L59, L60, L65, L67, L69, L76, L78, L79, L80, L82	L05, L10, L15
T2	81419 Soybean	L05, L06, L07, L08, L09, L11, L13, L14, L15, L17, L18, L19, L20, L24, L27, L28, L29, L30, L31, L32, L33, L35, L38, L40, L41, L42, L43, L44, L45, L46, L47, L49, L51, L53, L54, L55, L56, L59, L60, L63, L65, L66, L69, L71, L76, L77, L78, L79, L80	L25, L79

4.5.4 Laboratories not providing a quantitative result

A large proportion (41 % for MON88302 and 31 % for 81419) of the 74 participants in this CT round did not quantify one or both GM events, hence their performance for analysis of these events could not be evaluated.

Table 9 lists the participants that failed to perform quantification of the GM events identified in the test items, which was one of the required tasks in this CT round. Two of these participants provided a semi-quantitative result in the form of a value above a threshold value.

Table 9. Participants to comparative test ILC-EURL-GMFF-CT-02/15 that failed to quantify oilseed rape event MON88302 (T1) and/or soybean event 81419 (T2).

Test Item	GM Event	No Quantitative Result Submitted	Semi-quantitative Result Provided
T1	MON88302 Oilseed Rape	L02, L03, L11, L12, L14, L18, L21, L22, L23, L25, L26, L34, L43, L44, L48, L51, L57, L58, L61, L63, L64, L66, L68, L71, L72, L73, L74, L77	L39, L54
T2	81419 Soybean	L01, L02, L03, L10, L12, L21, L22, L23, L26, L34, L36, L37, L39, L48, L57, L58, L61, L64, L67, L68, L73, L74, L82	

The reasons for the failure of many laboratories to submit quantitative results are unknown. For T1, this may be related to the complexity of the matrix and the difficulty of obtaining suitable DNA from this matrix, together with the requirement for quantification of an oilseed rape GM event, which is not a common test in many laboratories. For T2, the GM event to be quantified was a rather new event listed under Regulation 619/2011 as an event for which the authorisation in the EU is pending.

Nevertheless, control laboratories, and in particular the NRLs, should be able to identify and quantify all events that could be present in food and/or feed entering the European market.

5. Conclusions

Participants in this CT round were required to analyse two test items varying in composition and complexity. For test item T1, the 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 powdered instant soup comprising several vegetable species and contained a low proportion of oilseed rape, which was increased to approximately 4.5 % by addition of non-GM oilseed rape flour and MON88302 oilseed rape flour. Test item T2 was composed of a more uniform matrix (ground soybean corresponding to ERM-BF437d), containing 9.9 g/kg 81419 soybean.

The species present in the T1 matrix, and the MON88302 oilseed rape event, were correctly identified by the majority of participants. The evaluation of the quantitative results for this event resulted in three laboratories receiving an unsatisfactory z-score for quantification of oilseed rape event MON88302. Ninety-three percent of the laboratories performed satisfactorily for the GM event quantification in this complex test material.

In T2, all participants that tested for the soybean event 81419 correctly identified it, and 86 % of these also quantified the event. The quantitative results received for soybean event 81419 were satisfactory for all but two of these participants (96 %). Considering those laboratories that reported a quantitative result, this is a good outcome for a GM event that was only recently included in the EU register listing the GM events that fulfil the requirements of Regulation (EC) No 619/2011 and for which the validated detection method was only published a few months before the start of this CT round.

A large proportion of participants have not reported a quantitative result for one or both GM events to be tested and the performance of these laboratories was therefore not evaluated. These laboratories are strongly 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 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.

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

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
BG	National Center of Public Health and Analyses		Sofia
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	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
PT	INIAV		Lisboa
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

[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.

COUNTRY	ORGANISATION	DEPARTMENT	CITY
	CATEGORY b		
DE	Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	Jena
DE	BfR	Food Safety	Berlin
DE	LTZ Augustenberg		Karlsruhe
DE	LALLF MV	Dezernat 200, PCR	Rostock
DE	Landesamt für Verbraucherschutz Sachsen-Anhalt		Halle
DE	Institute for Hygiene and Environment		Hamburg
DE	Bavarian Health and Food Safety Authority (LGL)		Oberschleissheim
DE	CVUA Freiburg	GMO	Freiburg
DE	Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Amtliche Lebensmitteluntersuch	Dresden
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 EVIRA		Helsinki
IT	CRA-SCS	Sede di Tavazzano, Laboratorio	Tavazzano (LO)
NL	Netherlands Food and Consumer Product Safety Authority (NVWA)	Consument en Veiligheid	Wageningen
UK	Fera		York
UK	Scottish Government	SASA	Edinburgh
	CATEGORY c		
AR	Biotechnology Institute-CICVyA-INTA	GMO Laboratory Detection	Hurlingham-Bs. As.
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
CL	Servicio Agrícola y Ganadero	Biotechnology Laboratory	Santiago
CO	National Institute for Food and Drug Surveillance - INVIMA	OLCC	Bogotá
DE	CVUA RRW	FG 40-5	Krefeld
DE	Landesamt fuer Umweltschutz	FG13	Halle (Saale)
DE	Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	Jena
HK	Government Laboratory, HKSAR		Hong Kong
HR	Croatian Centre for Agriculture, Food and Rural Affairs	Biotechnological Analyses Divi	Osijek
HU	BIOMI Ltd		Gödöllő
IN	ICAR-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
MX	SENASICA	CNRDOGM	Tecámac
PL	Institute of Biochemistry and Biophysics PAS		Warszawa
RO	Central Laboratory for Quality of Seeds and Planting Material Bucharest	LEDOMG	Bucuresti
RS	SP Laboratorija A.D.	Genetical and physico-chemical	Bečej
RS	A Bio Tech Lab	Laboratory for biotechnology	Sremska Kamenica
TR	Ankara Food Control Laboratory	Biogenetics	Ankara
TR	National Food Reference Laboratory	Biotechnology and GMO Unit	Ankara
UA	State scientific research institute of laboratory diagnostic and veterinary sanitary expertise	GMO detection	Kyiv
UK	Worcestershire Scientific Services		Worcester
VN	Agricultural Genetics Institute	GMO Detection Laboratory	Hanoi

Annex 1: Homogeneity and stability of test items

A1.1 Homogeneity of test items

Homogeneity of test item T2 has been demonstrated as part of the certification of ERM-BF437d by IRMM. The assessment of the homogeneity[16] 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[17] 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[18].

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-fold 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 related to the level of homogeneity of T1.

A1.2 Stability of test items

For T2, the short-term stability was confirmed as part of the certification of ERM-BF437d confirming that the material remains stable during shipment conditions and for at least one year after purchase.

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 [19].

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 oilseed rape event MON88302.

The test items were 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	18	71	10	6
Absent	56	1	63	62
Not tested	0	2	1	6

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	0	9	0	41
Maize 1507	1	1	13	11	9
Maize 3272	0	0	16	7	11
Maize 40278	2	0	6	13	15
Maize 5307	1	0	13	10	12
Maize 59122	0	0	15	1	18
Maize Bt11	0	0	16	8	9
Maize GA21	0	0	13	7	12
Maize MIR162	0	0	13	7	10
Maize MIR604	0	0	15	8	11
Maize MON810	4	1	11	13	6
Maize MON863	0	0	15	9	10
Maize MON87460	0	0	16	6	12
Maize MON88017	0	0	13	8	13
Maize MON89034	0	0	15	8	11
Maize NK603	0	1	14	9	10
Maize T25	1	1	14	8	10

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 GM soybean events tested	1	0	8	1	45
Soybean 305423	2	0	6	10	15
Soybean 356043	2	0	10	10	11
Soybean 40-3-2	0	0	14	8	10
Soybean 68416	0	0	10	9	15
Soybean 81419	0	0	11	6	9
Soybean 44406	0	0	9	4	19
Soybean A5547	0	0	13	7	12
Soybean A2704	0	0	13	7	12
Soybean CV127	0	0	13	7	12
Soybean FG72	1	0	6	10	15
Soybean MON87701	1	0	6	12	13
Soybean MON87705	1	0	7	9	15
Soybean MON87708	1	0	7	9	15
Soybean MON87769	1	0	6	10	15
Soybean MON89788	3	0	7	11	13

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	3	1	0	1	9
OSR 73496	3	1	5	42	24
OSR GT73	14	2	5	54	7
OSR MON88302	30	50	0	0	14
OSR MS8	0	0	0	0	0
OSR RF3	0	0	38	22	12
OSR T45	1	1	37	23	11

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	0	1	23	5	46

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 81419	7	56	0	1	14
Soybean 68416	3	2	2	59	12
Soybean MON89788	1	0	15	61	5

Q1.7. Did you test the DNA extracts for the occurrence of PCR inhibition?	T1	T2
a) We have not tested for PCR inhibition	15	12
b) We run two or more dilutions and verify the delta Ct	16	15
c) We run two or more dilutions and verify the final GM % are similar	0	38
d) We compare the measured Ct of the undiluted extract to the Ct extrapolated from a dilution series	8	11
e) In another way (please specify)	9	9

Q1.8. Please specify how PCR inhibition was tested, if answered e) in previous question.

We compare Cp of the undiluted positive control with Cp of the diluted sample in control positive (dilution factor 1/2)
Test for PCR efficiency, r-squared and extrapolated Ct as per the JRC Guidance document "Verification of analytical methods for GMO testing when implementing interlaboratory validated methods"
We always look at the curves and compaire to reference material
TaqMan® Exogenous Internal Positive Control kit used
Inhibition controls in the PCR run (samples + positive control-DNA)
We performed the amplification of the reference gene of the sample and compared the Cp value with a positive control wich belonged to a RM
Eurofins GMO Screening kit includes IPC (Internal positive control).
Undiluted samples and 4x dilutions were run and verifed the delta Ct.

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

Q2.1. Where did you obtain the DNA extraction method from?	T1	T2
a) ISO/CEN published method	17	17
b) EURL validated method	7	6
c) National reference method	1	1
d) International literature	1	1
e) In-house developed	6	6
f) Commercial kit	46	45

Q2.2. Which DNA extraction method or extraction kit did you use?	T1	T2
a) CTAB method	29	28
b) SDS method	2	2
c) Biotecon	3	3
d) GeneScan GeneSpin	5	5
e) Guanidine HCl	1	1
f) Macherey-Nagel NucleoSpin	18	15
g) Promega Wizard	3	3
h) Qiagen DNeasy plant mini kit	3	5
i) Qiagen DNeasy Mericon Food kit	2	1
j) Other	13	16

Q2.3. Please specify the DNA extraction method or kit, if not listed.	Number of Laboratories
Maxwell 16 FFS NA Extr. System (Promega)	4
Fast ID Genomic DNA Extr. kit	2
Generon Ion Force	2
SureFood Prep Advanced	2
Qiagen EZ1 DNA Tissue kit	1
Qiagen DNeasy Blood & Tissue kit (modified)	1
Phenol-chloroform method	1
Promega micro food feed and seed kit	1
DNA Extraction Kit (GMO and Allergen) NEOGEN is a new name for TEPNEL kit.	1

Q2.4.Was the DNA further cleaned-up following use of the method specified above?	T1	T2
a) No additional DNA clean-up	49	50
b) Additional ethanol precipitation	8	8
c) Eurofins DNAExtractor cleaning column	4	4
d) Promega Wizard DNA clean-up resin	4	3
e) Qiagen QIAQuick	2	2
f) Qiagen Genomic-Tip 20/G	0	0
g) Other (no need to specify)	7	6

Q3. How many replicate DNA extractions were used to obtain the quantitative result(s) reported?	T1	T2
b) 2	37	38
c) 3	8	8
d) 4	8	10
e) 5	2	1
f) 6	1	0
g) >6	1	0

Q4. Which general approach was used to analyse the test items?	T1	T2
a) Three-step analysis: screening - event identification - event quantification	39	18
b) Two-step analysis: screening - event quantification	2	2
c) Two-step analysis: event identification - event quantification	2	29
d) Two-step analysis: screening + event identification, then event quantification	5	4
e) One-step analysis: event quantification	0	0
f) No quantification was performed	9	4

Q5. Which real-time PCR instrument was used for quantification (not for qualitative analysis)?	Number of Laboratories
a) No real-time PCR instrument was used	1
b) ABI 7000	0
c) ABI 7300	6
d) ABI 7500	21
e) ABI 7700	0
f) ABI 7900 (HT)	11
g) ABI ViiA7	2
h) ABI StepOne & StepOne Plus real-time PCR system	2
i) BioRad iCycler (iQ)	2
j) BioRad CFX	4
k) ABI QuantStudio	2
l) Qiagen/Corbett Rotor-Gene	2
m) Roche LightCycler 480	7
n) Roche LightCycler 1.2	0
o) Roche LightCycler 2.0	2
p) Stratagene Mx	7
q) Other	1

Q5.1. If other, please specify.	Number of Laboratories
AriaMx Realtime PCR System, Agilent	1

Q6. Which event-specific methods were used for determining the quantitative result(s)?	T1	T2
a) Reference method from EURL GMFF GMOMETHODS database	40	48
b) Reference method from other database	0	0
c) National reference method	0	0
d) ISO/CEN method	0	0
e) In-house developed and optimised	2	2
f) International literature	0	0
g) Commercial quantification kit (e.g. GeneScan)	0	0
h) No quantification was performed	21	14

Q7. Which endogenous target DNA sequence was used as taxon-specific reference gene for quantification?	T1	T2
No quantification was performed	16	15
Soybean lec 74 bp	7	42
Soybean lec 80 bp	2	2
Soybean lec 81 bp	1	4
Soybean lec 102 bp	0	1
Soybean lec 105 bp	0	2
Soybean lec 118 bp	0	0
Oilseed rape CruA	28	3
Olseed rape BnC1	5	0
Oilseed rape FatA(A)	4	0
Oilseed rape HMGa	2	0
Rice PLD	6	0
Rice GOS9	0	0
Rice SPS	7	1
Maize hmg	2	1
Maize adh1-70 bp	2	1
Maize adh1-134 to 136 bp	2	0
Maize zSSIIb/zein/ivr	0	0
Other	14	0

Q8. How were the final quantitative results determined?

Q8.1. Which reference material was used for calibration?	T1	T2
CRM from IRMM, certified for GM mass fraction (g/kg)	2	50
CRM from IRMM, certified for GM DNA copy number ratio (plasmid calibrant)	0	0
CRM from AOCS, certified for GM presence (purity)	42	4
Non-certified RM (e.g. QC material), expressed in GM mass fraction	0	0
Non-certified RM, expressed in GM DNA copy number ratio (e.g. determined by digital PCR)	0	0
No quantification was performed	26	19

Q8.2. Test Item 1 (T1): Please specify the reference material used for calibration (e.g. ERM-BF413gk, AOCS 0407B, ...)	T1
AOCS 1011-A	40

Q8.3. Test Item 2 (T2): Please specify the reference material used for calibration (e.g. ERM-BF413gk, AOCS 0407B, ...)	T2
ERM-BF437 series	49

Q8.4. Was a conversion factor used to translate cp/cp% into m/m%?	T1	T2
No conversion necessary, all data are in m/m %	36	45
GM event is homozygous, cp/cp % is same as m/m %	4	6
GM event is hemizygous, conversion factor (copies to mass) was derived from EURL validation report	1	0
Conversion was done based on digital PCR performed in my lab	0	0
Conversion was done by multiplying the cp/cp % value by a factor of 2	5	0
No quantification was performed	26	19

Q8.5. Test Item 1 (T1): Specify the actual conversion factor used, if applicable (otherwise write NA).	T1
NA	42
2	5

Q8.6. Test Item 2 (T2): Specify the actual conversion factor used, if applicable (otherwise write NA).	T2
NA	46

Q9. How was the measurement uncertainty determined?	T1	T2
a) No quantification was performed	27	21
b) From repeatability SD	28	29
c) From reproducibility SD	12	13
d) In another way	5	6

Q10. Additional comments
Soy 81419 no reference material available, so not tested for T2.
The quantification for oilseed rape events was performed by the Landesamt für Umweltschutz Saxony-Anhalt, due to legal requirements in Saxony-Anhalt.
Relative standard deviation (ISO21570:2005)
Only traces of maize and soybean (Ct>35) were detected in Test Item 1 (T1).
Primers and probe for soybean lec taxon-specific reference gene were from the method QT_GM_005 (84bp)
it was not possible to quantify the rapeseed T1 sample as this isn't a substrate we have been called on to quantify before and we could not justify the expense of the standards and primer/probe sets for this alone.
We do not have the necessary reagents for testing 81419 and 44406 soybean
For the T1 sample, the oilseed rape cruciferin Ccf endogenous reference system has been used
Potato (UGP) detected in Test Item 1
GT73 in T1 is found at very low level (Ct~39-40) in 3 out of 4 extracts=>not quantifiable; Quantification 81419, MON 88302 out of accreditation. Ref system for MON 88302 - ccf 78 bp
Quantification was not performed due to non-availability of reference standards in oilseed rape and rice (for test sample 1) and soybean (for test sample 2). In the experimenets for maize quantification, amplification of 135 bp region of Adh1 in the test sample 1 was not consistent so quantification experiments were not conducted.
Type B uncertainty
% GM was calculated using 2 different DNA extractions of samples and CRM on two different days (different analysts), giving 8 measurements in total
First comment to question 7.: We used Cruciferin ccf of the reference method from EURL-GMFF MON88302 as taxon-specific gene for quantification; Second comment: according to respective contracts in North-Rhine-Westphalia the specification and quantification of OSR events were carried out in the State Institute of Chemical and Veterinarian Analysis Eastwestphalia-Lippe (CVUA-OWL)
Trace amount of maize was detected in T1. MU from EURL interlaboratory validation relative reproducibilty standard deviation at 0.9% with k=2
c) within-laboratory reproducibility
Uncertainty=coverage factor (P=95%, f=n-1) * standard deviation / square-root(number of measurements) . We used for oilseed rape the taxon-specific reference gene PEP.
Question 7: Phosphoenyl-Pyruvate-Carboxylase (PEPCase) gene as reference target for oilseed rape (T1)
For Test Item 1 (T1) was made an event-specific PCR that showed a positive result for CruAOilseed rape (CT 25.94) and UGPase Potato (CT 21.07), but was not made a quantitative analysis for technical reasons.
DNA extraction, screening PCR and event identification for both samples was done by our Lab. Event quantification of T1 was done by our Lab too, but event quantification of T2 was done by Landesamt fuer Verbraucherschutz Saxony-Anhalt because of official regulated Lab cooperation in Saxony-Anhalt.
Expressed as % GMO based on the formula used for the measurement uncertainty = 2*%RSD/2.83*2.5*Xmean/100
In item 1 only canola species was identified and the only available test event. In item 2 the only event that is available is the MON89788 has.
p.1.2: towards analysis request for test item 1 and 2, flow analysis concerns first species identification, then GMO screening analysis through 6 markers detection. On the basis of obtained result patterns we filled this form, reporting the event presumptive presence when specific screening markers were detected and no qualitative event specific analysis was operated in lab.
Reference materials are used as positive controls, as no quantification was performed
Item 1 (instant soup) : amplification plots observed for soybean and mayze species Real time PCR assays, but considered as below LOD
In the internal method for determining uncertainty we take in account reproducibility and Bias
For T1 we identified p35S, pFMV and for T2 item as negative by screening. Within the framework this analyse and plant screening we just worked on the event that includes p35S and pFMV systems by event spesific PCR for T1.For T2 item, 68416, 81419 soybean events are not in our scope. We didn't analyse these events.
- Question 1.6: T2 was tested for pat gene & found positive, thus SB 68416 or SB81419 or both of them may be present. - i) Testing for the presence of OSR 73496, ii) further testing of T2, iii) quantification of GM events present in both T1 & T2 cannot be currently carried out in our laboratory, due to a limited availability of reagents (delays of public procurement process of reagents).
Q. 7: For oilseed rape there is no option for Ccf as reference gene. Ccf reference gene was used for quantification of MON 88302 according to method EURL-VL-09/11VR.

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.

For T1, the approach relies on the calculation of z-scores from $\log_{10}$-transformed data[20,21] 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) for T1 on both original and $\log_{10}$-transformed scale, taking into account the agreed standard deviation ($\hat{\sigma}$) for comparative testing, set to 0.2 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:

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

For T2, corresponding to ERM-BF437d, the z-scores were calculated relative to the certified value of this CRM (CV_{CRM}), using a standard deviation of 0.15, as agreed by the Advisory Board. The formula used was as follows:

$$z_i = \left(\log_{10} x_i - \log_{10} \mathrm{CV}_{\mathrm{CRM}}\right) / \hat{\sigma} \qquad \text{(A3.2)}$$

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-02/15 (- = not available).

Laboratory Code	Test Item 1			Test Item 2		
	MON88302 Oilseed Rape (μ_R = 1.16 m/m %)			81419 Soybean (Assigned Value = 0.99 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L06	1.30	0.54	0.4	1.09	0.18	0.3
L08	-	-	-	0.70	0.29	-1.0
L09	1.29	-	0.3	0.95	-	-0.1
L10	0.29	0.04	-2.9	-	-	-
L13	0.98	0.46	-0.2	1.06	0.37	0.2
L24	1.84	0.31	1.1	1.19	0.17	0.5
L29	1.53	0.75	0.7	0.82	0.16	-0.5
L30	0.85	0.35	-0.6	0.87	0.31	-0.4
L32	0.80	0.3	-0.7	0.85	0.32	-0.4
L33	1.30	0.4	0.4	0.92	1.03	-0.2
L35	1.32	0.37	0.4	0.88	0.25	-0.3
L38	1.04	-	-0.1	0.75	-	-0.8
L40	0.75	0.28	-0.8	0.83	0.16	-0.5
L42	1.35	0.27	0.4	0.73	0.14	-0.9
L43	-	-	-	0.85	-	-0.4
L44	-	-	-	0.90	0.22	-0.3
L51	-	-	-	0.99	0.08	0.0
L53	0.43	0.91	-2.0	0.79	0.27	-0.7
L54	-	-	-	0.95	-	-0.1
L56	1.16	0.06	0.1	1.05	0.12	0.2
L59	1.27	0.43	0.3	1.09	0.14	0.3
L60	1.03	0.30	-0.1	0.90	0.27	-0.3
L65	1.08	0.48	0.0	0.97	0.17	-0.1
L66	-	-	-	0.99	0.29	0.0
L69	1.81	0.54	1.1	0.83	0.25	-0.5
L77	-	-	-	0.78	0.27	-0.7
L78	0.71	0.21	-0.9		0.37	0.6
L80	0.87	0.18	-0.5		0.16	-0.2
L82	1.49	0.33	0.7		-	-

Table A4.2. Performance of "Category b" laboratories (NRL/120) in comparative test ILC-EURL-GMFF-CT-02/15 (- = not available).

Laboratory Code	Test Item 1			Test Item 2		
	MON88302 Oilseed Rape (μ_R = 1.16 m/m %)			81419 Soybean (Assigned Value = 0.99 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L05	0.34	0.03	-2.5	1.10	0.56	0.3
L07	1.55	0.31	0.7	0.84	0.10	-0.5
L08	1.50	0.45	0.7	-	-	-
L17	1.56	0.10	0.8	0.75	0.05	-0.8
L19	0.90	-	-0.4	1.05	0.07	0.2
L28	1.23	0.46	0.2	0.89	0.1	-0.3
L31	1.34	0.08	0.4	1.16	0.12	0.5
L36	0.99	0.18	-0.2	-	-	-
L41	1.62	0.67	0.8	1.15	0.33	0.4
L45	0.60	0.18	-1.3	0.97	0.29	-0.1
L46	2.05	0.25	1.4	1.05	0.03	0.2
L47	1.33	-	0.4	0.87		-0.4
L49	1.88	0.64	1.2	1.11	0.48	0.3
L63	-	-	-	1.53	0.35	1.3
L71	-	-	-	1.17	0.15	0.5
L76	1.06	0.19	-0.1	0.93	0.04	-0.2

Table A4.3. Performance of "Category c" laboratories (non-NRL) in comparative test ILC-EURL-GMFF-CT-02/15 (- = not available).

Laboratory Code	Test Item 1			Test Item 2		
	MON88302 Oilseed Rape (μ_R = 1.16 m/m %)			81419 Soybean (Assigned Value = 0.99 m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m%)	Uncertainty (m/m %)	z-score
L01	1.82	0.14	1.1	-	-	-
L11	-	-	-	0.56	1.04	-1.6
L14	-	-	-	1.07	20.08	0.2
L15	0.16	-	-4.2	0.66	-	-1.2
L18	-	-	-	0.9	0.26	-0.3
L20	0.64	0.11	-1.2	0.98	0.14	0.0
L25	-	-	-	5.14	-	4.8
L27	0.71	0.12	-0.9	1.05	0.15	0.2
L37	1.38	0.40	0.5	-	-	-
L55	1.93	0.55	1.2	0.86	0.09	-0.4
L67	0.97	0.30	-0.3	-	-	-
L72	-	-	-	1.00	-	0.0
L79	0.60	0.18	-1.3	0.25	0.10	-4.0

Figure A4.1. Z-scores for oilseed rape event MON88302 in Test Item 1 on the basis of a robust mean of 1.16 m/m % (◊).

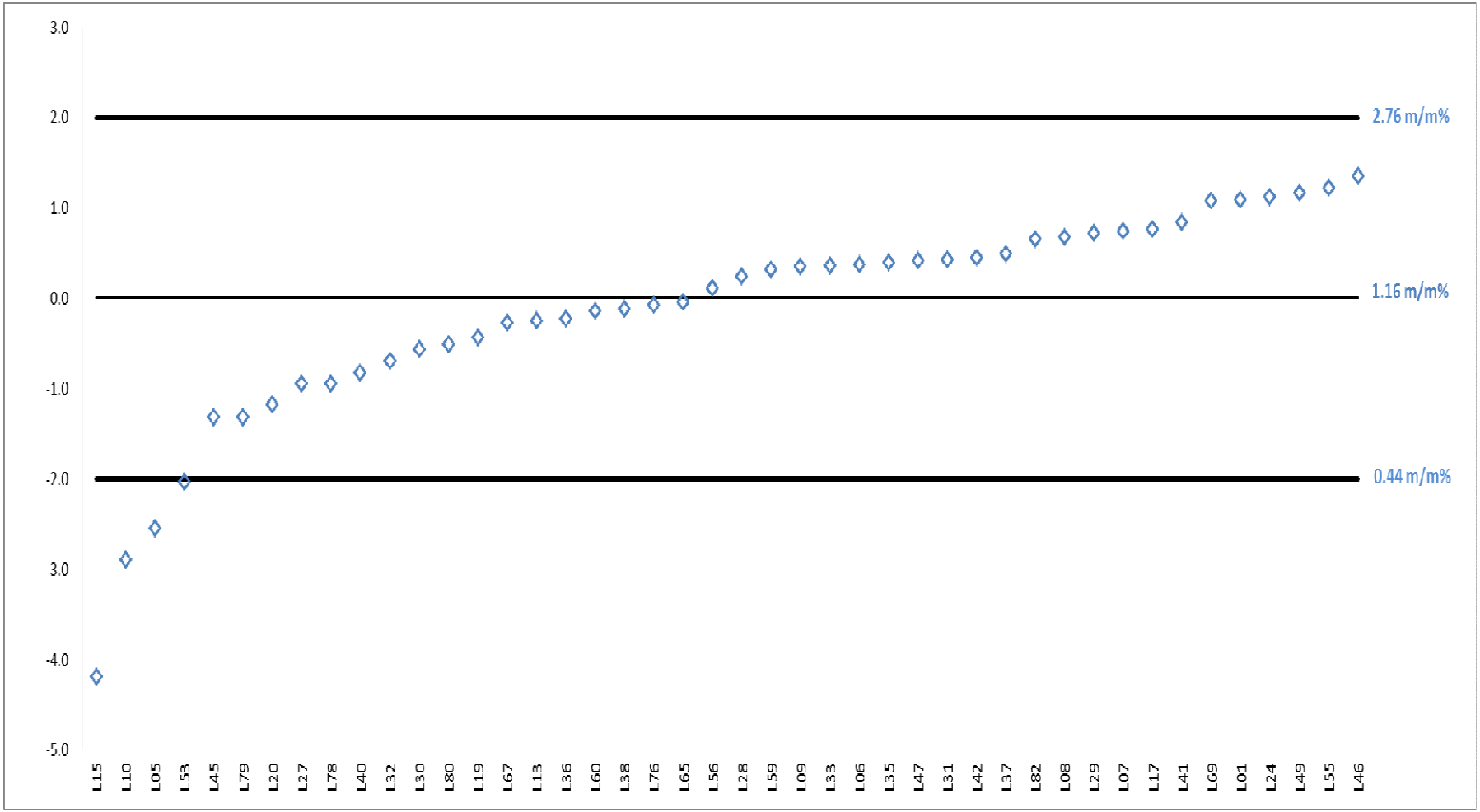
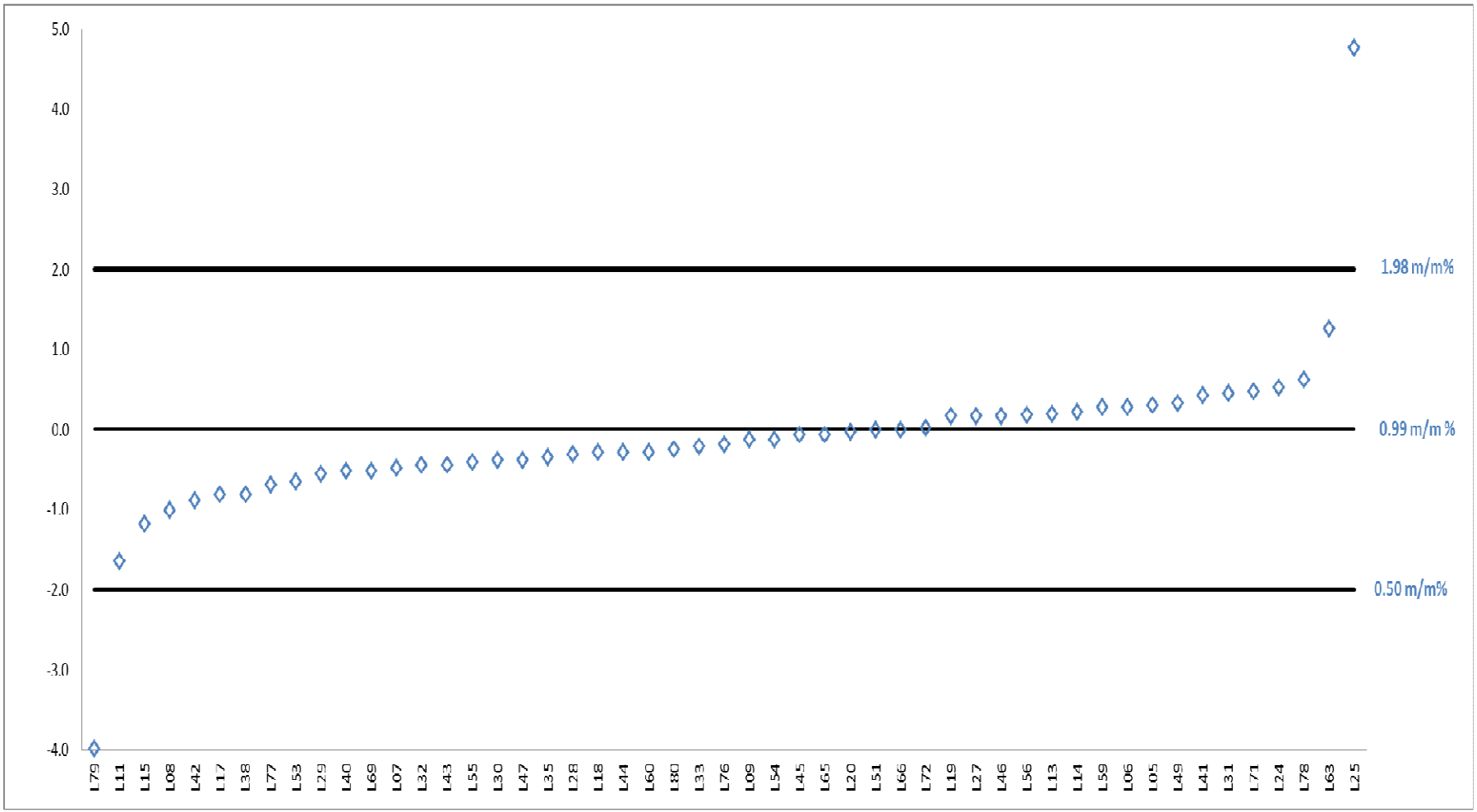


Figure A4.2. Z-scores for soybean event 81419 in Test Item 2 on the basis of the assigned value of 0.99 m/m % (◊).



Annex 5: Invitation letter

Ref. Ares(2015)2196374 - 27/05/2015

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

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 26 May 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-02/15

Dear Colleague,

Hereby, I would like to invite you to participate in the 12th round of comparative testing ILC-EURL-GMFF-CT-02/15, 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: "Instant soup"
- Perform species identification (maize, soybean, oilseed rape and rice);
- Identify and quantify the GM event(s) detected.

Test Item 2: "Feed, Soybean"
- Screen for the presence of the following three GM soybean events: MON 89788, 68416, 81419;
- 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.

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

ISO 9001:2008 certified by
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=1461

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 **Wednesday 10 June 2015**. Samples will be shipped during the week of **15 June 2015**. The deadline for submission of the results is **Friday 21 August 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,

p.o.

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

Annex 6: Accompanying letter to shipment of samples

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

European Union Reference Laboratory
for GM Food & Feed

JRC.DG.I.3/MBG/JK/wb/lv/ARES(2015) 2512565

NOTE FOR THE ATTENTION OF

All Laboratories registered for the comparative test ILC-EURL-GMFF-CT-02/15

Viviana Pedroarias (L67)
Biotechnology Institute-CICVyA-INTA
De Los Reseros
y Nicolás Repetto
1686 Hurlingham-Bs. As.
ARGENTINA

Subject: **ILC-EURL-GMFF-CT-02/15, a comparative testing round to determine the GM content in two test materials, *i.e.* a food and a soybean feed.**

Dear Dr Pedroarias,

Thank you for participating in the ILC-EURL-GMFF-CT-02/15 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 as scanned pdf. You should store the samples in a dark and cold place (not exceeding 4 °C).

Tasks
Participants should:

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

Test Item 2: "Feed, Soybean"
- Screen for the presence of the following three GM soybean events: MON 89788, 68416, 81419;
- 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:

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

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

ISO 9001:2008 certified by

JRC.I3.T50_EURL_1 CT - yyyy-mm-dd_Participation in ILC-EURL-GMFF-CT-XXYY

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 PVBG1167411. The system will guide you through the reporting procedure.

After entering all results, please complete the questionnaire online (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 on-line on the reporting website https://web.jrc.ec.europa.eu/ilcReportingWeb **will be accepted.**

Directly after submitting your results and the questionnaire information on-line, you will be prompted to print the completed report form. Please sign the printed report form and return it to the EU-RL 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 EU-RL GMFF will not verify whether your data are complete.

The deadline for submission of results is Friday 21 August 2015. 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)2523756

Dear Participant,

Your test parcels related to the 12th comparative testing round ILC-EURL-GMFF-CT-02/15 left our premises today, **16 June 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 accompanying letter**.**

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

Your Lab Code («LCode») is indicated in the accompanying letter; please keep it for future uses in this CT round.

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

Via separate e-mail it will be sent:

- The questionnaire (which will need to be filled in online on the reporting website)
- An "acknowledgement of reception" form, that should be returned, **fully filled and signed,** to the EU-RL GMFF, as scanned pdf, by e-mail to mbg-comparative-testing@jrc.ec.europa.eu;

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 **fax: +39 0 332 78 6159**
Method Validation / EURL-GMFF
European Commission - Joint Research Centre - IHCP
21027 ISPRA (VA) Italy

File nb EURL-CT-02/15

We have received the following samples **In good condition** **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

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

References

1. European Commission (2003). Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Off. J. Eur. Union* L 268: 1-23
2. European Commission (2004). Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Union* L 191: 1-52
3. ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing
4. European Commission (2011). Commission Regulation (EU) No 619/2011 of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorisation procedure is pending or the authorisation of which has expired. *Off. J. Eur. Union* L 166: 9-15
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