

JRC TECHNICAL REPORTS

Determination of GM Oilseed Rape 73496 and GT73 in Rapeseed Cake and GM Soybean MON89788 in Soybean Flour

*Comparative testing
ILC-EURL-GMFF-CT-02/16*

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Quality assurance

The European Union Reference Laboratory for GM Food and Feed (EURL GMFF) is accredited according to ISO/IEC 17025:2005 (accreditation number: ACCREDIA 1172) for the testing of food and feed (flexible scope) for GMOs (DNA extraction, detection, identification and quantification by PCR).

The EURL GMFF is also accredited according to ISO/IEC 17043:2010 (accreditation number: ACCREDIA 0012) for the organisation of proficiency tests (here called comparative tests or CT).



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

COUNTRY	ORGANISATION	DEPARTMENT	CITY
CATEGORY ¹ a			
AUSTRIA	Umweltbundesamt GmbH		Vienna
AUSTRIA	AGES-Institute for Food Safety Vienna		Vienna
BELGIUM	Institute for Agricultural and Fisheries Research	Technology and Food - PI	Merelbeke
BELGIUM	Centre Wallon de Recherches Agronomiques	Valorization of agric. prod.	Gembloux
BELGIUM	Scientific Institute of Public Health (WIV-ISP)	PBB - GMolab	Brussels
BULGARIA	National Center of Public Health and Analyses	GMO Unit	Sofia
CROATIA	Croatian Institute of Public Health		Zagreb
CYPRUS	State General Laboratory	GMOs and Allergens	Nicosia
CZECH REPUBLIC	Crop Research Institute		Prague
DENMARK	Danish veterinary and Food Administration	Food Chemistry and Plant health	Ringsted
FINLAND	Finnish Customs Laboratory	ET2	Espoo
FRANCE	SCL - Service Commun des Laboratories		Illkirch
FRANCE	BioGEVES		Surgeres
GERMANY	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	Referat 503	Berlin
HUNGARY	National Food Chain Safety Office		Budapest
ITALY	Istituto Zooprofilattico Delle Regioni Lazio e Toscana	Biotechnology Unit	Rome
LATVIA	Institute of Food Safety, Animal Health and Environment „BIOR“		Riga
LITHUANIA	National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO	Vilnius
LUXEMBOURG	Laboratoire National de Santé	Surveillance Alimentaire	Dudelange
NETHERLANDS	RIKILT Wageningen University & Research	NFA	Wageningen
POLAND	Regional Laboratory of Genetically Modified Food		Tarnobrzeg
POLAND	National Veterinary Research Institute	Feed Hygiene	Pulawy
POLAND	Instytut Zootechniki PIB KLP Szczecin		Szczecin
PORTUGAL	INIAV	UEIS-SAFSV	Oeiras
ROMANIA	Institute for Diagnosis and Animal Health	Molecular Biology and GMOs	Bucharest
SLOVAKIA	State Veterinary and Food Institute Dolny Kubin		Dolny Kubin
SLOVAKIA	Central Control and Testing Institute of Agriculture	OMB NRL	Bratislava
SLOVENIA	National Institute of Biology		Ljubljana
SPAIN	Laboratorio Arbitral Agroalimentario LAA-MAGRAMA	OGM	Madrid
SPAIN	Centro Nacional De Alimentación (Agencia Española De Consumo, Seguridad Alimentaria Y Nutrición)	Biotechnology Unit	Madrid
SWEDEN	Livsmedelsverket (National Food Agency)	Biology	Uppsala
UNITED KINGDOM	LGC		Teddington

COUNTRY	ORGANISATION	DEPARTMENT	CITY
CATEGORY b			
FINLAND	Finnish Food Safety Authority Evira		Helsinki
GERMANY	CVUA Freiburg		Freiburg
GERMANY	LAVES-LVI Braunschweig/Hannover	FB12	Braunschweig
GERMANY	Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	Halle
GERMANY	Landeslabor Berlin-Brandenburg (LLBB)	Fb I-6	Berlin
GERMANY	Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Amtliche Lebensmitteluntersuchung	Dresden
GERMANY	Landeslabor Schleswig-Holstein		Neumünster
GERMANY	LTZ Augustenberg		Karlsruhe
GERMANY	Thüringer Landesamt für Verbraucherschutz	Lebensmittelsicherheit	Bad Langensalza
GERMANY	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei	Mecklenburg- Vorpommern	Rostock
GERMANY	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)		Oberschleissheim
GERMANY	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GB 6, Fachbereich 63	Nossen
ITALY	CREA-SCS	Sede di Tavazzano, Laboratorio	Tavazzano (LO)
NETHERLANDS	NVWA. Netherlands Food and Consumer Product Authority	Laboratorium VV	Wageningen
POLAND	Plant Breeding and Acclimatization Institute NRI	GMO Controlling Laboratory	Blonie
UNITED KINGDOM	Fera Science Ltd (Fera) ²	04F10	York
UNITED KINGDOM	SASA		Edinburgh
CATEGORY c			
ARGENTINA	Instituto de Biotecnología CICVyA INTA	Laboratorio de OGM	Hurlingham
BELGIUM	FASFC Melle	GMO	Melle
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BRAZIL	Ministry of Agriculture Livestock and Food Supply	LANAGRO-GO	Goiania-Goiás
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CROATIA	CCAFRA, Institute for Seed and Seedlings	Biotechnological Analyses Divi	Osijek
GERMANY	Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	Jena
GERMANY	Landesamt fuer Umweltschutz Sachsen-Anhalt	FG13	Halle (Saale)
GERMANY	Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)		Detmold
HONG KONG	Government Laboratory, HKSAR	Government Laboratory	Hong Kong
HUNGARY	Biomi Kft.	GMO labor	Godollo
INDIA	ICAR-National Bureau of Plant Genetic Resources, New Delhi	Division of Genomic Resources	New Delhi
INDONESIA	National Quality Control Laboratory of Drug and Food	Biotechnology Laboratory	DKI Jakarta
ITALY	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta	S.C. Biotecnologie	Torino
ITALY	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna		Brescia
MEXICO	SENASICA	CNRDOGM	Tecamac
PHILIPPINES	Bureau of Plant Industry, National Plant Quarantine Services Division, Post Entry Quarantine Station	Department of Agriculture	Los Banos, Laguna
POLAND	Institute of Biochemistry and Biophysics PAS		Warszawa
POLAND	Wojewodzki Inspektorat Weterynarii	Zaklad Higieny Weterynaryjnej	Opole
SERBIA	SP Laboratorija a.d.	Genetical dpt.	Becej
SERBIA	A Bio Tech Lab	Laboratory for Biotechnology	Sremska Kamenica
SINGAPORE	Agri-Food & Veterinary Authority	Veterinary Public Health Centr	Singapore
SWITZERLAND	Federal Food Safety and Veterinary Office FSVO	Risk Assessment Division	Bern

COUNTRY	ORGANISATION	DEPARTMENT	CITY
CATEGORY c (continued)			
SWITZERLAND	Agroscope		Posieux
TURKEY	National Food Reference Laboratory	Biotechnology and GMO Unit	Ankara
UKRAINE	Ukrainian Laboratory of Quality and Safety of Agricultural Products		Chabany village
UKRAINE	Ukrmetrteststandart	Molecular biology	Kiev
UNITED KINGDOM	Worcestershire County Council	Scientific Services	Worcester
UNITED STATES	USDA-GIPSA	Biotechnology Laboratory	Kansas City
VIETNAM	QUATEST 3	Microbiology - GMO Lab	Ho Chi Minh City
VIETNAM	National Institute for Food Control (NIFC)	GMO lab	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.

² Fera also participated on behalf of the NRL designated by Ireland under Regulation (EC) No 882/2004.

Abstract

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF), accredited according to ISO/IEC 17043, organised a comparative testing (CT) round for National Reference Laboratories (NRLs) to support the official controls on food and feed in line with Regulation (EC) No 882/2004. Other official control laboratories were allowed to participate on a voluntary basis.

Two test items were distributed: a rapeseed cake material spiked with oilseed rape GM events 73496 and GT73 (Test Item 1, T1) and a feed sample composed of soybean flour containing soybean event MON89788 (Test Item 2, T2). Participants were required to screen T1 and T2 for the presence of three GM oilseed rape events and three GM soybean events, respectively, and to quantify those events identified. The results had to be reported in GM mass/mass %.

Eighty-one participants from 39 countries participated to this CT round, including 49 NRLs, of which 32 are designated in line with Regulation (EC) No 882/2004 (NRL/882) and 17 are nominated in Regulation (EU) No 120/2014 to support the EURL GMFF on method validation (NRL/120).

The qualitative results, i.e. the correct identification of the GM events, were evaluated and scored as correct or incorrect. The quantitative results were compared to the consensus value of the data provided by the NRLs determined by robust statistics. A z-score was calculated and scored unsatisfactory if $|z| > 2.0$.

The qualitative results reported by the NRLs indicated that all had identified the correct GM events in both test items. Also the quantitative results were mainly satisfactorily.

A total of 20 laboratories obtained an unsatisfactory result for the quantification of one or more GM events, including 7 out of the 32 NRL/882 and 5 out of the 17 NRL/120. Follow-up actions will be organised for the NRLs with an unsatisfactory outcome for one or more GM events in this CT round.

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 coordinating the official controls to ensure the verification of compliance with food and feed law. The analytical methods used for these controls have been validated by the EURL GMFF, as required by Regulation (EC) No 1829/2003, and for this task, the EURL GMFF is supported by NRLs listed in Regulation (EU) No 120/2014⁽³⁾ (NRL/120; a part of these NRL/120 are also NRL/882). The Member States of the European Union may also appoint other official control laboratories (non-NRLs) for performing the official controls on food and feed.

It is crucial that official control laboratories can accurately and reliably determine the GM content in food and feed samples. Regulation (EC) No 1829/2003 established a threshold for labelling of food and feed products containing genetically modified material that is authorised in the EU (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 genetically modified material with pending or expired authorisation status. Compliance with these values is verified by the Member States of the European Union in the official control of food and feed.

The EURL GMFF is tasked with the organisation of comparative testing (CT) to foster the correct application of the analytical methods available for the official controls⁽²⁾. The EURL GMFF is accredited according to ISO/IEC 17043⁽⁵⁾ for the organisation of proficiency testing.

This report summarises the results obtained in the second CT round organised by the EURL GMFF in 2016 (CT 02/16). Participation in these CT rounds is mandatory for NRL/882, recommended for NRL/120, and open to official control laboratories 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. The EURL GMFF prepared and characterised the test items, managed the online registration of participants, evaluated the results reported by the participants and assessed their performance. This activity is supported by experts from the Advisory Board for Comparative Testing.

2 Test items

The T1 test item was prepared by the EURL GMFF from base materials that were characterised before their use (Table 1). The T2 test item was identical to the T2 used in CT round 01/14 (Table 2). The bottles of T2 were re-labelled with a sample number and the description "Sample T2 (Feed)".

The base materials employed for the preparation of T1 were ground to a powder where necessary and the water content was determined by an oven drying method. The DNA extractability was verified as follows: using the standard CTAB and Macherey-Nagel NucleoSpin Food methods low amounts of DNA were recovered from some of the base materials. Two other extraction methods were therefore tested (Table 1): the CTAB method developed by DuPont Pioneer, owner of the GM event 73496, and validated by the EURL GMFF for event 73496, and the Foodproof Sample Preparation Kit III (Biotecon Diagnostics GmbH, Potsdam, Germany). The latter method was chosen for all further analyses because it yielded a good amount of DNA without problems with PCR inhibition. Note, however, that with this Biotecon method the DNA extractability from the non-GM oilseed rape material was significantly larger than from the other base materials. By gel electrophoresis, the rapeseed cake DNA appeared largely fragmented (smear from ± 25 to 1 kbp), whereas the DNA extracted from the other base materials ran as a single band ≥ 25 kbp. The presence of unexpected GM events in the base materials was tested by using event-specific pre-spotted plates⁽⁶⁾. All base materials were lacking unexpected GM events, except for a low amount of GT73 (Cq 36.7) detected in the non-GM oilseed rape flour.

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

- Because of a limited mass of rapeseed cake material non-GM oilseed rape flour was added to a final concentration of 18.9 m/m % (Table 1);
- The masses of the GM ingredients to add (73496 and GT73) were calculated taking into account their water content (Table 1);
- The compound sample was manually mixed for 10 min, then thoroughly mixed for 60 min in a Turbula T10B mixer.
- The T1 mix was used to prepare 150 test items containing 5 g of flour in 30-ml bottles using a sample divider (Retsch GmbH, Haan, DE), which were then labelled with a sample number and the description "Sample T1 (Feed)".
- All test items were stored at 4 °C.

Homogeneity and stability testing of T1 was performed in-house, as described in Annex 1, using event-specific quantification methods previously validated by the EURL GMFF. Material T1 was found to be homogeneous for both GM events (p -value > 0.05 ; 200 mg sample intake). The average measured concentrations for event 73496 (0.41 m/m %) and GT73 (0.30 m/m %) in T1 were found to be somewhat lower than expected on the basis of the gravimetric preparation; this was confirmed by droplet digital PCR (0.41 and 0.20 m/m % for 73496 and GT73, respectively) and may be due to the higher extractability of the non-GM oilseed rape DNA compared to the DNA from the other materials. Also the impact of the former processing of the rapeseed cake, and of the resulting degraded DNA (as evidenced by gel electrophoresis), on the final GM quantification is unknown. As the assigned value will be calculated as the robust mean of the participants' results, the deviations from the target gravimetric values do not have a consequence for this CT exercise.

From the isochronous study, it was concluded that the test item would be sufficiently stable under ambient shipment conditions (5 % significance level – See Annex 1).

Table 1. Characteristics of the base materials used for preparation of test item 1 (T1).

Characteristic	Rapeseed cake	Oilseed rape (OSR)	73496 OSR	GT73 OSR
Type of base material	Rapeseed cake	Non-GM oilseed rape	Flour from 73496 (purity 100 %) used to produce ERM-BF434 ⁽⁸⁾	CRM AOCS 0304-B2 (Pure, homozygous GT73/RT73 canola) ⁽⁹⁾
Origin	Market sample provided by L. Houghs (DK)	AOCS 0304-A	IRMM	AOCS
Grinding method	Retsch GM200	Retsch GM200	NA	Retsch GM200
Water content in m/m %, mean \pm SD ($n = 10$)	8.20 \pm 0.17	6.25 \pm 0.19	4.30 \pm 0.23	4.34 \pm 0.24
DNA extractability in ng/mg¹, mean \pm SD ($n = 10$)	C: 0.33 \pm 0.03 B: 0.46 \pm 0.11	C: 0.69 \pm 0.11 B: 1.34 \pm 0.11	C: 0.64 \pm 0.06 B: 0.53 \pm 0.12	C: 0.43 \pm 0.06 B: 0.46 \pm 0.03
GM events detected with event-specific pre-spotted plates²	None	Traces of GT73 (Cq 36.7)	None ²	GT73
Mass used to prepare T1 (g)	607.63	143.47	4.39	3.66
Nominal target GM concentration in T1 (m/m %)	NA	NA	0.6	0.5

¹ Sample intake was 200 mg for both CTAB (C) (DuPont Pioneer method validated for 73496) and Biotecon (B).

² An all-species event-specific pre-spotted plate (PSP) was used for all tests; the PSP version used did not contain the 73496 method. NA = not applicable; SD = standard deviation.

Table 2. Characteristics of test item 2 (T2).

Characteristic	Soybean feed
Type of base material	Ground soybean flour spiked with MON89788 soybean flour
Origin	Re-used test item 2 of CT 01/14 containing MON89788 soybean (robust mean 0.89 m/m % based on 52 results reported in this unit); see Report ILC-EURL-GMFF-CT-01/14 for details on the preparation and characterisation

Homogeneity and short-term stability of T2 had been previously demonstrated as part of CT 01/14. Stability (on the longer term) was re-confirmed by analysis of three extracts each from two bottles stored at 4 °C and one bottle stored at the reference temperature (-70 °C). A two-sample *t*-test assuming equal variances revealed the absence of a significant difference between the results obtained on bottles stored at 4 °C and -70 °C, thereby confirming the stability of the test item.

3 Instructions to participants

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

For Test Item 1, "Rapeseed cake" (feed):

- Screen for the presence of the following three GM oilseed rape events: 73496, GT73 and MON88302;
- Quantify the GM oilseed rape event(s) detected.

For Test Item 2, "Soybean flour" (feed):

- Screen for the presence of the following three GM soybean events: 44406, MON87701 and MON89788;
- Quantify the GM soybean event(s) detected.

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

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

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 %, while results obtained using a calibrant certified for DNA 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⁽¹⁰⁾.

4 Results

4.1 Participation to CT round 02/16

On 14 September 2016, 165 laboratories were invited to participate in the CT round ILC-EURL-GMFF-CT-02/16 and 83 laboratories subsequently registered for it and received a random unique lab code (L01 to L83). Eighty-one laboratories from 39 countries returned results within the reporting deadline. Two laboratories did not submit any results, one of which (L54) reported not being able to perform the whole analysis in time due to the heavy workload in the laboratory. Table 3 shows an overview of the participation to this CT round.

Table 3. Invitation and participation to the comparative testing round CT 02/16.

Characteristic of the CT round	Result
Date of invitation	14 September 2016
Number of invited laboratories	165
Number of registered laboratories	83
Date of shipment of samples	4 and 5 October 2016
Deadline for result submission	17 November 2016
Registered laboratories that failed to submit their data	L54, L57
Number of participating laboratories	81

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

- Thirty-two NRLs designated in line with Regulation (EC) No 882/2004 (NRL/882), representing 25 EU Member States (many of these are also NRL/120). In addition, Ireland is delegating its NRL/882 tasks to one of the CT participants. Greece (due to internal re-organisation), Estonia and Malta were not represented in this CT round.
- Seventeen NRLs nominated under Regulation (EU) No 120/2014 (NRL/120) who are not at the same time official control laboratories under Regulation (EC) No 882/2004.
- Thirty-two official control laboratories, but not NRLs nominated under either of the Regulations mentioned above. This category includes 12 EU laboratories and 20 laboratories from non-EU countries, including Serbia and Switzerland.

Table 4. Overview of participants to CT 02/16 by country and category.

Country	Participants	NRL/882	NRL/120	Non-NRL
ARGENTINA	1			1
AUSTRIA	2	2		
BELGIUM	4	3		1
BRAZIL	2			2
BULGARIA	2	1		1
COLOMBIA	1			1
CROATIA	2	1		1
CYPRUS	1	1		
CZECH REPUBLIC	1	1		
DENMARK	1	1		
FINLAND	2	1	1	
FRANCE	2	2		
GERMANY	15	1	11	3
HONG KONG	1			1
HUNGARY	2	1		1
INDIA	1			1
INDONESIA	1			1
ITALY	4	1	1	2
LATVIA	1	1		
LITHUANIA	1	1		
LUXEMBOURG	1	1		
MEXICO	1			1
NETHERLANDS	2	1	1	
PHILIPPINES	1			1
POLAND	6	3	1	2
PORTUGAL	1	1		
ROMANIA	1	1		
SERBIA	2			2
SINGAPORE	1			1
SLOVAKIA	2	2		
SLOVENIA	1	1		
SPAIN	2	2		
SWEDEN	1	1		
SWITZERLAND	2			2
TURKEY	1			1
UKRAINE	2			2
UNITED KINGDOM	4	1	2	1
UNITED STATES	1			1
VIETNAM	2			2
Total	81	32	17	32

4.2 Information on the testing provided in the questionnaire

Participants were asked to fill in an online questionnaire (through EUSurvey) on their testing methodology for T1 and T2, consisting of a number of mostly multiple-choice questions. A total of 78 laboratories completed the questionnaire (L20, L23, and L43 did not submit the questionnaire). Table 5 summarises the main answers received, except the GM identification results which are reported in Section 4.3; Annex 2 shows all answers.

Table 5. Summary of the main answers provided in the questionnaire of CT 02/16.

Question	Test Item 1	Test Item 2
Test item analysed	Yes (91 % ¹), No (9 %)	Yes (99 %), No (1 %)
DNA extraction method	CTAB (45 %), NucleoSpin Food (15 %)	CTAB (47 %), NucleoSpin Food (13 %)
Additional DNA purification method	None (63 %), Ethanol (10 %)	None (68 %), Ethanol (10 %)
Number of replicates	2 (64 %), 4 (12 %)	2 (69 %), 4 (13 %)
Approach to test for PCR inhibition	OD ratios (46 %), delta Cq or GM % between two dilutions (31 %)	OD ratios (49 %), delta Cq or GM % between two dilutions (36 %)
Reason for not testing all events	Not applicable (44), reagents not available (18)	Not applicable (57), reagents not available (13)
Approach used	73496: standard curves (39), delta Cq (8) GT73: standard curves (56), delta Cq (8)	MON89788: standard curves (60), delta Cq (8)
Calibrant used	73496: CRM IRMM in m/m % (59 %), no answer (41 %) GT73: pure CRM AOCS (81 %), no answer (19 %)	MON89788: pure CRM AOCS (83 %), no answer (14 %)
Taxon-specific endogenous gene	73496: <i>CruA</i> (27 %), <i>FatA(A)</i> (27 %) GT73: <i>CruA</i> (71 %), <i>FatA(A)</i> (3 %)	MON89788: <i>lec</i> -74 bp (74 %), other <i>lec</i> targets (total 12 %)
Unit of measurement and data expression	73496: Mass (33), copies=mass CRM (12) GT73: Mass (43), copies=mass CRM (14)	NA ²
Amount of DNA	73496: 100 ng (17), 50 or 200 ng (11) GT73: 200 ng (23), 100 ng (17)	MON89788: 100 ng (20), 200 ng (17)
LOQ determination	73496: EURL (22), current analysis (19) GT73: in-house validation (25), EURL (19)	MON89788: in-house validation (27), current analysis (21)
Reason for lack of analysis	Matrix out of scope (4), methods not validated (3)	Matrix out of scope (1), no answer (77)

¹ The percentages shown are per total number of answers received including blanks; if a number is given, this refers to the number of laboratories reporting this answer. Generally, the answers that were reported with the two largest frequencies are mentioned.

² This question was not requested for T2 because soybean is homozygous, therefore conversion between units does not create issues.

An evaluation of the answers showed that the most commonly employed DNA extraction method for both T1 and T2 was one based on CTAB, with the NucleoSpin Food kit ranking second. Additional purification methods were generally not applied. In most cases two replicate DNA extracts were analysed. Only 1 in 6 laboratories (13 out of 78) performed a PCR inhibition run on 3 or 4 DNA dilutions with a reference gene before analysis. Nine laboratories tested 3 or 4 DNA dilutions and verified if the delta Cq or GM % were as expected, and one in three laboratories ran two dilutions for the same purpose. Almost half of the laboratories only checked the quality of the DNA extracts by verifying the OD ratios, and about half of these also monitored the profile of the amplification curves. One (T1) and three (T2) laboratories only relied on experience for excluding PCR inhibition without assessing it.

For the quantitative analysis, the most common approach used was based on two standard curves. Despite the fact that only the 73496 detection method was validated with a delta Cq approach by the EURL GMFF, the same number of laboratories (8) used such an approach for both 73496 and GT73, and also for MON89788. One laboratory (L31) mentioned the use of digital PCR as additional method for verification of their qPCR

results. The CRM from IRMM or AOCS corresponding to the GM event were used by all laboratories, except two non-NRLs (L58 and L64) who used a non-certified reference material for MON89788 quantification. *CruA* was used as taxon-specific reference gene by most laboratories for both oilseed rape events, and the 74 bp *lec* gene target for MON89788 soybean. Forty-two to 55 % of laboratories performed their measurements in the same unit as the certified value of the calibrant used (m/m %), whereas 15-18 % used DNA copies in their calculation sheets, but assumed that 10 m/m % equalled 10 copy/copy % (see further below). Many laboratories did not answer the question on how the LOQ was determined for each of the GM events; among the answers received, roughly 1/3 calculated it from the data of the current CT, 1/3 during in-house method validation, and 1/3 took it from the EURL validation report (approximation over the three GM events). In most cases a LOQ of 0.1 m/m % or lower was reported.

4.3 GM event identification

Table 6 summarises the results reported by the participants through the questionnaire regarding the (qualitative) identification of the GM events. Note that the answers provided in the questionnaire were sometimes incomplete and do not always match up with what could be deducted from the quantitative results (e.g. in some cases the identification of an event was not reported, while for the same event a quantitative result was provided). Three laboratories had not filled in the questionnaire. The numbers reported in Table 6, which are only based on the data reported by the participants in the questionnaire, therefore, do not necessarily match with the overall evaluation provided further in this report.

One NRL/882 reported that T1 was out of the scope of the laboratory, as agreed between the NRLs within the Member State and communicated to the EURL GMFF; in this case a sister NRL/882 in the same Member State provided results for this sample.

Among the NRLs, the large majority of laboratories identified the correct events in T1 (73496 and GT73) and T2 (MON89788). Some laboratories also identified other GM events: L17 reported detection (no quantification) of MON88302 in T1 and L52 detected all three events, MON89788, 44406 and MON88701, in T2 (without quantification of the latter two). This reporting of unexpected GM events in a test item is considered less important regarding compliance with the labelling rules as it is presumed that only traces of these were detected, below the technical solution for labelling of feed under Regulation (EU) No 619/2011. Although none of the NRLs reported the absence of any of the events that should have been detected in T1 or T2, a number of them did not test for some of the events and could therefore not confirm their presence or absence; the unavailability of the reagents for these assays was given as major reason for not having performed the tests.

The results of the non-NRLs were also correct in most cases, however, a larger proportion of laboratories had not tested all GM events, and three laboratories had reported the absence of an event that should have been identified.

Table 6. GM event identification results of the participants as reported in the questionnaire, expressed in number of laboratories.
(Note: for unexpected results the lab code is provided within brackets)

Laboratories	Test Item	GM Event	Present	Absent	Not Tested	Sample Not Analysed
NRL/882 and NRL/120	T1	73496	43	0	4	1
		GT73	47	0	0	
		MON88302	1 (L17)	42	2	
	T2	44406	1 (L52)	42	2	1
		MON87701	1 (L52)	44	0	
		MON89788	47	0	0	
Non-NRLs	T1	73496	12	1 (L04)	6	6
		GT73	22	1 (L58)	0	
		MON88302	0	12	6	
	T2	44406	1 (L58)	13	10	0
		MON87701	1 (L51)	18	6	
		MON89788	27	1 (L76)	1	

Note: the totals do not necessarily sum up to the total number of participating laboratories as some laboratories have not filled in the questionnaire or have not (correctly) answered all questions.

4.4 GM event quantification

4.4.1 Quantitative results reported by the participants

Of the 81 laboratories that participated to this CT round, the number of participants that submitted event-specific quantitative data for each of the GM events present in the test items is shown in Table 7. A significant proportion of laboratories had not quantified event 73496 oilseed rape, and some had not quantified one or both of the two other events. Additionally, a number of laboratories reported a value "smaller than" (<) or "higher than" (>); one non-NRL (L04) had reported a value of <0.01 % for 73496, one NRL/882 (L42) had reported a value of 0 % for GT73.

The performance of those laboratories that had not reported a quantitative result for one or more of the events was not evaluated. However, the performance of NRL/882 participants, who should have been able to quantify all GM events, was considered unsatisfactory in case they had not reported a quantitative result (see below).

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

Quantitative Results Reported	Test Item 1		Test Item 2
	73496 OSR	GT73 OSR	MON89788 Soybean
Number of laboratories reporting a quantitative result	47	64	69
Number of laboratories reporting the measurement uncertainty	39	52	57
Number of laboratories reporting the coverage factor used	32	44	49

A measurement uncertainty was reported for 82 % of all measurement results, with the coverage factor reported for 69 % of the results. These percentages are similar to those in previous CT rounds. Among the NRL/882, all but 6 laboratories (L09, L14, L29, L45, L62 and L71) systematically provided a measurement uncertainty for every result reported.

All participants are reminded that a measurement result is only complete if it is accompanied by a statement on the uncertainty of the measurement result. To emphasise the importance of correctly reporting results, the uncertainties reported will be taken into account for the data evaluation in future CT rounds.

4.4.2 Assigned values

The assigned values for events 73496 and GT73 in T1, and MON89788 in T2, were based on the consensus values (μ_R) from the data from participants in this CT round, calculated using robust statistics^(12,13). This approach minimises the influence of outlying values. The data taken into account for calculation of the robust means were those from the NRLs (NRL/882 and NRL/120) only. The data from the non-NRLs were excluded because of the heterogeneity of this group, some laboratories being experienced in GMO analysis, others not.

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

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

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

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

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

Assigned Values & Uncertainties	Test Item 1		Test Item 2
	73496 OSR	GT73 OSR	MON89788 Soybean
Type of assigned value	Robust mean, μ_R	Robust mean, μ_R	Robust mean, μ_R
Number of data points (NRLs)	37	44	46
$u_{char, rel}$ (%)	7.95	7.43	3.75
$u_{bb, rel}$ (%)	2.78	4.95	3.37
Assigned Value (m/m %)	0.50	0.30	0.82
Expanded Uncertainty ($U = 2*u$)	0.09	0.06	0.09

The robust means for the events spiked into the rapeseed cake (T1) were slightly lower than the gravimetrically calculated GM percentages, and this may be due to the higher extractability of the non-GM oilseed rape flour added to the mix.

For T2, the robust mean calculated for event MON89788 (0.82 ± 0.09 m/m %) is in agreement with the value calculated in CT 01/14 (0.89 ± 0.09 m/m %).

4.4.3 Calculation of z-scores

To evaluate laboratory performance, z-scores were calculated on the basis of the assigned value and the target standard deviation for each event (see Annex 3, formula A3.1). This was done for all laboratories who had provided a quantitative result, including non-NRLs. The target standard deviations were fixed by the Advisory Board for Comparative Testing at 0.2 for T1 and 0.15 for T2 (log scale), in line with the complexity of the test item matrix, and taking into account the results of previous CT rounds. For consistency, all decimal numbers were rounded to two digits.

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

A total of 24 quantitative results, reported by 20 laboratories, resulted in an unsatisfactory z-score, half of which (12) were for event GT73 in T1. Four laboratories obtained an unsatisfactory result for two events, two of these got it for both events in T1. Seven out of the 32 NRL/882 obtained an unsatisfactory outcome for one or more of their reported results, and 5 out of the 17 NRL/120.

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

Laboratory Performance	Test Item 1		Test Item 2
	73496 OSR	GT73 OSR	MON89788 Soybean
Assigned value μ_R	0.50	0.30	0.82
Lower z-score limit	0.18	0.11	0.40
Upper z-score limit	1.17	0.72	1.61
Number of laboratories with a satisfactory z-score	41	52	63
Number of laboratories with an unsatisfactory z-score	6	12	6

The results of L61 (non-NRL) were remarkable in that a >10 times too high concentration (3.94 m/m %) was reported for GT73 in T1; the same laboratory had performed the quantitative analysis for this event on DNA extracted by L31 and L72 (personal communication) and these values were also too high (3.28 m/m %). It seems therefore that there has been a problem with the calibration of the measurements or there has been a typing or calculation error.

L31, in addition to qPCR, had also informally communicated the results of digital PCR measurements for MON89788 in T2. With both techniques the concentration determined was 1.02 m/m %, which was satisfactory.

Two different taxon-specific reference targets are commonly used for relative quantification of oilseed rape GM events: *CruA* and *FatA(A)*. It should be noted that *CruA* occurs in two copies in the oilseed rape genome, while *FatA(A)* (developed by DuPont Pioneer for event 73496) is a single copy target on the A-genome of *Brassica*. Two laboratories have employed an older *FatA* assay developed by Monsanto; this particular *FatA* target (76 bp), like *CruA*, seems to exist in two copies in the genome (P. Corbisier, personal communication).

4.5 Performance of the laboratories

The overall performance of the laboratories participating in this CT round was evaluated on the basis of both the qualitative (*i.e.* the correct identification of the GM events) and the quantitative results reported. A satisfactory performance outcome was attributed to those laboratories who had correctly identified the GM event and obtained a satisfactory z-score for its quantification. The laboratories who had not tested a GM event or those who had identified the event but had not reported a quantitative value were not

considered as overall satisfactorily performing. While individual laboratories may have a valid reason for not analysing a certain GM event, the overall satisfactory performance score provides an estimate of the capacity of the participants in this CT round to adequately detect and quantify each of the three GM events. The results of the evaluation are shown per laboratory and laboratory category in Annex 5, Tables A5.1 to A5.3. A summary is provided in Table 10.

Table 10. Summary of the performance of laboratories for identification and quantification of the GM events in CT 02/16.

Performance Evaluation (%)	Laboratory Category	GM Identification			GM Quantification		
		T1		T2	T1		T2
		73496	GT73	MON89788	73496	GT73	MON89788
Good performance in % of those who tested the event	NRL/882	100	100	100	86	86	90
	NRL/120	100	100	100	93	73	93
	Non-NRL	93	96	97	80	80	91
Good performance in % of all participants ¹	NRL/882	87	100	100	61	81	88
	NRL/120	100	100	100	82	65	88
	Non-NRL	52	92	94	32	64	66

¹ All participants means all those that participated except those that had reported not having tested the sample.

The results revealed a satisfactory performance of the participants for the identification of the GM events, however, as many non-NRLs had not tested for event 73496, the percentage decreased to 52 % when expressed per total number of laboratories.

The performance for GM event quantification was acceptable for 73-93 % of participants per category; when also the laboratories that had not tested the event were included, this figure was reduced to 65-88 % for the NRLs and 32-66 % for the non-NRLs.

In CT 01/14, 7 unsatisfactory z-scores were reported for the quantification of MON89788 in T2. All of these laboratories, except two who did not participate in CT 02/16, obtained a satisfactory result for the same sample in the current CT. This shows that these laboratories have improved their performance over the recent years. The proportion of laboratories that did not analyse this event also decreased from 19 % in 2014 (14 participants) to 15 % (12 participants) now.

In case of an unsatisfactory performance the laboratories will be requested to perform a root cause analysis and to communicate the outcome to the EURL GMFF.

5 Conclusions

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

The results reported by the participants were analysed and a performance evaluation was carried out taking into account both the qualitative and the quantitative results reported and including the missing results; a failure to test or to quantify a GM event was considered unsatisfactorily in relation to the tasks of this CT round. The majority of the participants performed satisfactorily for all tasks in this CT round, *i.e.* the detection and quantification of the events 73496 and GT73 in T1, and MON89788 in T2. An unexpectedly large number of laboratories, 20 in total, including 7 NRL/882, obtained an unsatisfactory z-score for one or more GM events. More unsatisfactory z-scores were obtained for event GT73 (12) compared to 73496 (6) and MON89788 (6).

The performance of laboratories was in general better for T2 compared to T1, an observation in line with the complexity of the material to be analysed, as T1 was a processed feed material characterised by a low DNA quality, while T2 was (unprocessed) soybean flour. The concentration of the GM events was also different between T1 and T2: while the MON89788 concentration in T2 was close to the legal threshold for labelling, the concentrations of 73496 and GT73 were significantly below this threshold. The low GMO concentrations, and the processing effects seen in the rapeseed cake (T1), could have contributed to the relatively large number of unsatisfactory results obtained by the participants in this CT round.

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

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List of abbreviations

CT	Comparative testing (= proficiency testing)
EURL	European Union Reference Laboratory
GMFF	Genetically modified food and feed
m/m %	Mass per mass percentage
OSR	Oilseed rape
qPCR	Quantitative (real-time) Polymerase Chain Reaction

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Annexes

Annex 1. Homogeneity and stability of test items

A1.1 Homogeneity of test items

Homogeneity of test item T2 had been demonstrated previously and was reported in the final report of ILC-EURL-GMFF-CT-01/14 (see <http://gmo-crl.jrc.ec.europa.eu/Comparative-Testing.html>).

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:

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

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

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

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:

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

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

Seven bottles ($N = 7$) were randomly selected and analysed in five replicates ($n = 5$). The criterion described in formula (A1.1) was fulfilled, indicating that T1 was homogeneous. The data from the homogeneity study were also used for the estimation of the uncertainty contribution relating to the level of homogeneity of T1 (u_{bb} , see Table 8).

A1.2 Stability of test items

For T1, an isochronous short-term stability study involving two test samples with three replicates each ($N = 2$, $n = 3$) was conducted over two and four weeks at +4 °C, +18 °C and +60 °C ⁽¹⁹⁾. The 73496 oilseed rape concentration was measured and it was assumed that a similar matrix, oilseed rape event GT73, would evolve in the same way.

The results did not reveal any influence of time or storage at +4 °C or +18 °C on the stability of the test item (compared to storage at -70 °C) with regard to oilseed rape event 73496. At 60 °C a significant trend towards a reduced GM content was measured. Looking at the data and the graphics, the effect seems small and may not be real, e.g. two weeks storage at 4 °C gives a mean at the same level as 4 weeks storage at 60 °C. Also, when omitting the two lowest values at 4 weeks at 60 °C, the effect is not statistically significant anymore; similarly, when removing the largest value measured for 4 °C storage (0.585), the effect is gone. If the effect at 60 °C would be real, then it would appear only after two weeks of continuous storage at that temperature, which will rarely occur as the samples are generally delivered within two weeks. Monitoring of the results revealed that among the four participants (L26, L32, L40, L81) that had received the samples only between 2 and 3 weeks following distribution only one unsatisfactory z-score was obtained for GT73 in T1; as the result reported by this lab (L40) was too high, it was considered that it was unrelated to the stability of the material.


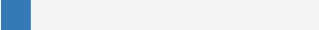
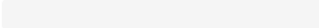
For T2, the short-term stability had been demonstrated previously and was reported in the final report of ILC-EURL-GMFF-CT-01/14 (see <http://gmo-crl.jrc.ec.europa.eu/Comparative-Testing.html>). As this test item had been stored at 4 °C for 2.5 years, its long-term stability was re-analysed by comparison of bottles stored at the normal storage temperature of 4 °C ($N = 2$, $n = 3$) with those stored at the reference temperature of -70 °C, ($N = 1$, $n = 3$). No significant difference in the MON89788 content (two-sample t-Test, 95 % confidence interval) was measured between either storage temperature, confirming that the material had remained stable.

The test items were shipped at ambient temperature.


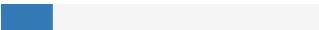
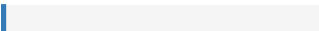
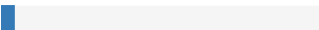
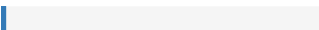
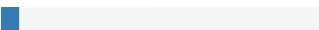
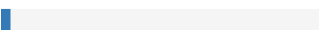
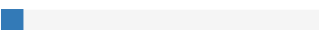
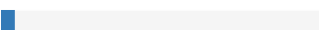
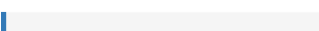
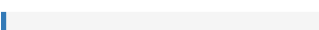
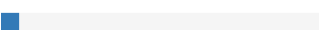
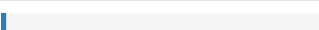
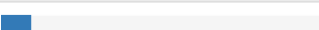

Annex 2: Questionnaire data

The results received from 78 laboratories were exported from the EUSurvey "Questionnaire on CT 02/16 analysis" and are tabulated below. Multiple answers were allowed for all questions, except for questions 7.b, 8.b and 9.b for both T1 and T2. The results of the open questions were manually analysed and reported.









T1: Please select the option that applies and proceed with the questionnaire (you may need to wait a few seconds before all additional questions open).

		Answers	Ratio
T1 was analysed: go to Q1		71	91.03 %
T1 was not analysed: go to Q10		7	8.97 %
No Answer		0	0 %

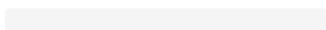







T1: 1. Select the DNA extraction method used for T1

		Answers	Ratio
CTAB		35	44.87 %
NucleoSpin Food		12	15.38 %
NucleoSpin Plant		1	1.28 %
GeneSpin		3	3.85 %
Promega Wizard		1	1.28 %
DNeasy Plant		4	5.13 %
DNeasy Mericon Food		2	2.56 %
Biotecon Foodproof		5	6.41 %
SDS		3	3.85 %
Fast ID Genomic DNA		1	1.28 %
Maxwell 16 Plant DNA		1	1.28 %
Maxwell 16 Food, Feed, Seed		4	5.13 %
Generon Ion Force		1	1.28 %
Other		7	8.97 %
No Answer		7	8.97 %










T1: 2. Select any additional DNA purification method used for T1.

		Answers	Ratio
No additional clean-up		49	62.82 %
Additional ethanol precipitation		8	10.26 %
Eurofins DNAExtractor cleaning column		2	2.56 %
Promega Wizard DNA clean-up resin		6	7.69 %
Qiagen QIAQuick		1	1.28 %
Qiagen Genomic-Tip 20/G		1	1.28 %
Other method (no need to specify)		4	5.13 %
No Answer		7	8.97 %

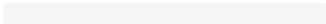


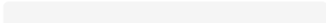
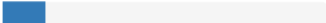
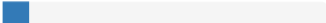
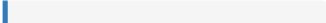
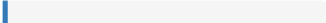
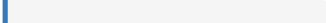

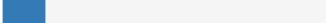
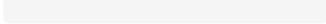
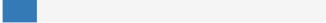
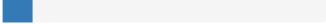
T1: 3. Indicate the number of replicate DNA extractions used to obtain the results.

		Answers	Ratio
1		0	0 %
2		50	64.1 %
3		4	5.13 %
4		9	11.54 %
5		3	3.85 %
6		2	2.56 %
>6		4	5.13 %
No Answer		7	8.97 %


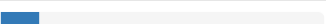

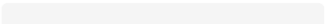
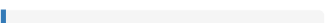


T1: 4. Select the approach(es) used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		2	2.56 %
We check that the optical density ratios (OD260/280, 260/230) are acceptable		36	46.15 %
We verify that the amplification curves look normal		21	26.92 %
We run two dilutions and verify if the delta Cq or GM% are as expected		24	30.77 %
We run three or four dilutions and verify if the delta Cq or GM% are as expected		10	12.82 %
We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq for undiluted extract, compare this to the measured Cq		16	20.51 %
We add an internal positive control to the reactions and check the Cq		9	11.54 %
Other		2	2.56 %
No Answer		7	8.97 %

T1: 5. Which GM events were detected and quantified in T1?

		Answers	Ratio
None		0	0 %
73496 was detected and quantified (Q7a-7g wil open)		46	58.97 %
GT73 was detected and quantified (Q8a-8g will open)		63	80.77 %
MON88302 was detected and quantified (Q9a-9g will open)		0	0 %
73496 was detected, but not quantified		10	12.82 %
GT73 was detected, but not quantified		6	7.69 %
MON88302 was detected, but not quantified		1	1.28 %
73496 was tested but found absent		1	1.28 %
GT73 was tested but found absent		1	1.28 %
MON88302 was tested but found absent		54	69.23 %
73496 was not tested		10	12.82 %
GT73 was not tested		0	0 %
MON88302 was not tested		8	10.26 %
No Answer		7	8.97 %

T1: 6. If applicable, why did you not test or quantify all GM events in T1?

		Answers	Ratio
a) Not applicable, all GM events listed were tested and all those detected were quantified		44	56.41 %
b) The event-specific detection method is not validated in our laboratory		9	11.54 %
c) Reference material, primers, probes, or other reagents were not available (in time)		18	23.08 %
d) The result obtained was below the LOD /LOQ		0	0 %
e) Practical constraints (instrument broken, no personnel, etc.)		1	1.28 %
f) Other reason		4	5.13 %
No Answer		7	8.97 %

T1: 7.a. Oilseed rape 73496: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		39	50 %
Delta Cq method (one calibration curve)		8	10.26 %
Digital PCR (no calibration curve)		0	0 %
No Answer		32	41.03 %

T1: 7.b. Select the calibrant used for the 73496 standard curve.

		Answers	Ratio
CRM from IRMM, certified in GM mass fraction (g/kg)		46	58.97 %
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction (g/kg or m/m %)		0	0 %
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		0	0 %
No calibrant used, digital PCR done		0	0 %
No Answer		32	41.03 %

T1: 7.c. Select the endogenous target(s) used for relative quantification of 73496 OSR in T1.

		Answers	Ratio
Oilseed rape CruA (from GT73 method)		21	26.92 %
Oilseed rape Ccf (from MON88302 method)		1	1.28 %
Oilseed rape FatA(A) - 126 bp (from 73496 method)		21	26.92 %
Other, please specify below		3	3.85 %
No Answer		32	41.03 %

Specify the reference target(s) used (if different from above):

L52 CruA, F- AAgAAGAA+TCA+TCA+TgC+T+TC--Q (+ means LNA)

L67 FatA – 76 bp - method from Monsanto

L78, L82 PEP – Phosphoenolpyruvate carboxylase - R. Zeitler, K. Pietsch, H.-U. Waiblinger (2002) Validation of real-time PCR methods for the quantification of transgenic contaminations in rape seed. Eur. Food Res. Technol., Volume 214, Number 4: 346 – 351.

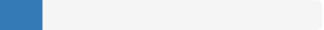
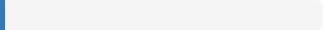
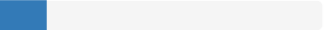
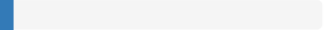
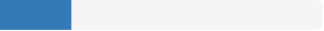
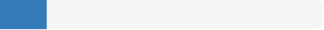
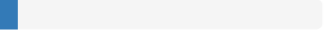
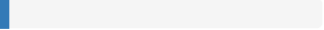
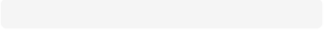
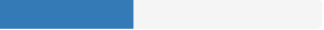
T1: 7.d. Clarify the unit of measurement used and any conversion between units if applicable. Carefully read the choices below and select the one used in the measurements that resulted in a final result in GM m/m % for 73496. If unclear or a different approach was used, please clarify this in the free text box below.

		Answers	Ratio
The RM and the calibration standards were expressed in mass (or mass %), no conversion factor was applied	<div><div></div></div>	33	42.31 %
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, but a conversion factor of 1 was applied (e.g. 10 % m/m GM = 10 % cp/cp GM, corresponding to a 10x dilution of a 100 % RM)	<div><div></div></div>	12	15.38 %
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, and a conversion factor >1 was applied to take account of the zygosity and target gene copies (double conversion applied); a conversion factor (e.g. : 2) was used to convert from mass to copies (e.g. 20 % m/m GM = 10 % cp/cp GM, corresponding to a 5x dilution of a 100 % RM); the final result was again converted to m/m % by using the same conversion factor (e.g. x 2). Please specify this factor below.	<div><div></div></div>	1	1.28 %
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). A conversion factor was applied onto the final GM %, please specify this factor below.	<div><div></div></div>	0	0 %
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). No conversion factor was applied onto the final GM %.	<div><div></div></div>	0	0 %
No Answer	<div><div></div></div>	32	41.03 %

Conversion factor used to turn results into m/m %, if applicable (73496) and/or clarification on preparation of standards.

L09 2 (answer option 3 selected to previous question)

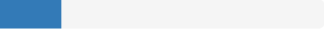
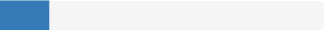
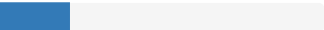
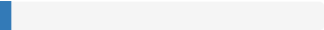
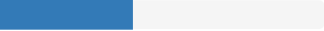
T1: 7.e. What was the amount of sample DNA (ng) used per PCR for 73496. Choose the concentration that is closest to what you used. If applicable, select multiple concentrations (e.g. if several dilutions were tested) but only those of which the result was used to determine the reported GM %.

		Answers	Ratio
DNA concentration not determined		10	12.82 %
250 ng		1	1.28 %
200 ng		11	14.1 %
150 ng		3	3.85 %
100 ng		17	21.79 %
50 ng		11	14.1 %
25 ng		4	5.13 %
15 ng		2	2.56 %
<10 ng		0	0 %
No Answer		32	41.03 %

T1: 7.f. What was the LOQ (in m/m %) for the 73496 quantification?

Value	Answers
0.01	1
0.02	3
0.04	1
0.05	5
0.06	1
0.08	8
0.09	1
0.1	13
No answer	45



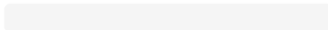

T1: 7.g. How was the LOQ for 73496 determined (if applicable)?

		Answers	Ratio
Determined from the qPCR analysis for the current sample		15	19.23 %
Determined during the in-house validation of the method		12	15.38 %
Taken from the EURL GMFF validation report		17	21.79 %
By another approach, please explain below		3	3.85 %
No Answer		32	41.03 %


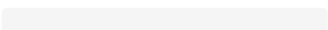
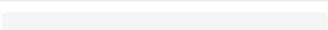


Explanation on alternative LOQ determination (73496):

L06	By default 0.1 %
L09, L36	Not determined






T1: 8.a. Oilseed rape GT73: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		56	71.79 %
Delta Cq method (one calibration curve)		8	10.26 %
Digital PCR (no calibration curve)		0	0 %
No Answer		15	19.23 %

T1: 8.b. Select the calibrant used for the GT73 standard curve.

		Answers	Ratio
CRM from AOCS, certified for GM presence (assuming 100% purity)		63	80.77 %
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction		0	0 %
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		0	0 %
No calibrant used, digital PCR done		0	0 %
No Answer		15	19.23 %


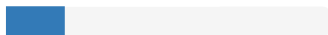
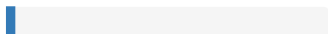
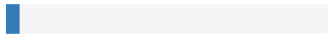
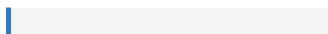
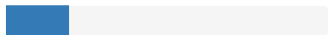
T1: 8.c. Select the endogenous target(s) used for relative quantification of GT73 OSR in T1.

		Answers	Ratio
Oilseed rape CruA (from GT73 method)		55	70.51 %
Oilseed rape Ccf (from MON88302 method)		1	1.28 %
Oilseed rape FatA(A) - 126 bp (from 73496 method)		2	2.56 %
Other, please specify below		5	6.41 %
No Answer		15	19.23 %

Specify the reference target(s) used (if different from above):

- L26 Rapeseed endogenous HMG i/y gene
- L52 CruA, F- AAgAAgAA+TCA+TCA+TgC+T+TC--Q (+ means LNA)
- L55, L67 FatA – 76 bp - method from Monsanto
- L78, L82 PEP – Phosphoenolpyruvate carboxylase

T1: 8.d. Clarify the unit of measurement used and any conversion between units if applicable. Carefully read the choices below and select the one used in the measurements that resulted in a final result in GM m/m % for GT73. If unclear or a different approach was used, please clarify this in the free text box below.

		Answers	Ratio
The RM and the calibration standards were expressed in mass (or mass %), no conversion factor was applied		43	55.13 %
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, but a conversion factor of 1 was applied (e.g. 10 % m/m GM = 10 % cp/cp GM, corresponding to a 10x dilution of a 100 % RM)		14	17.95 %
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, and a conversion factor >1 was applied to take account of the zygosity and target gene copies (double conversion applied); a conversion factor (e.g. : 2) was used to convert from mass to copies (e.g. 20 % m/m GM = 10 % cp/cp GM, corresponding to a 5x dilution of a 100 % RM); the final result was again converted to m/m % by using the same conversion factor (e.g. x 2). Please specify this factor below.		2	2.56 %
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). A conversion factor was applied onto the final GM %, please specify this factor below.		3	3.85 %
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). No conversion factor was applied onto the final GM %.		1	1.28 %
No Answer		15	19.23 %

Conversion factor used to turn results into m/m %, if applicable (73496) and/or clarification on preparation of standards.

L10 0.5 (answer option 2 selected to previous question)

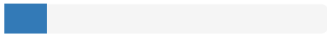
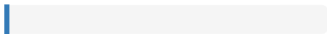
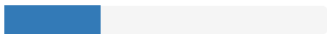
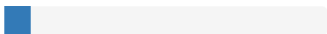
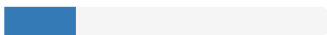
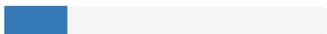
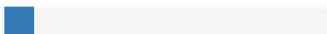
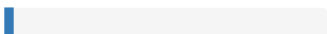
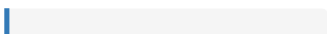
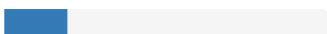
L26 1 (answer option 2 selected to previous question)

L09, L79 2 (answer option 3 selected to previous question)

L31, L61, L72 2 (answer option 4 selected to previous question)

L35 RT 73 is amphidiploid (CruA detects the A and the C genome). So here other quantification basis as for 73496! (answer option 1 selected to previous question)

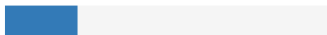
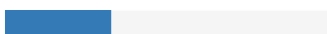
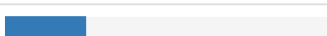
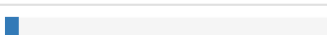
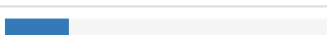
T1: 8.e. What was the amount of DNA (ng) used per PCR for GT73? Choose the concentration that is closest to what you used. If applicable, select multiple concentrations (e.g. if several dilutions were tested) but only those of which the result was used to determine the reported GM %.

		Answers	Ratio
DNA concentration not determined		10	12.82 %
250 ng		1	1.28 %
200 ng		23	29.49 %
150 ng		6	7.69 %
100 ng		17	21.79 %
50 ng		15	19.23 %
25 ng		7	8.97 %
15 ng		2	2.56 %
<10 ng		1	1.28 %
No Answer		15	19.23 %

T1: 8.f. What was the LOQ (in m/m %) for the GT73 quantification?

Value	Answers
0.01	1
0.02	1
0.03	1
0.04	1
0.05	4
0.06	1
0.07	1
0.08	5
0.09	4
0.1	22
0.32	1
0.87	1
No answer	35

T1: 8.g. How was the LOQ for GT73 determined (if applicable)?

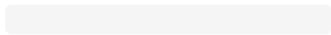
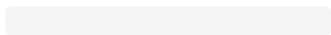
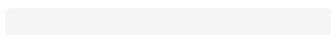

		Answers	Ratio
Determined from the qPCR analysis for the current sample		17	21.79 %
Determined during the in-house validation of the method		25	32.05 %
Taken from the EURL GMFF validation report		19	24.36 %
By another approach, please explain below		3	3.85 %
No Answer		15	19.23 %

Explanation on alternative LOQ determination (73496):

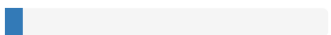
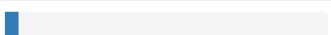
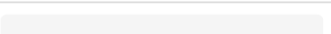
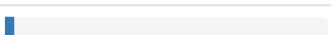


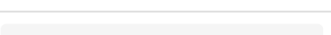

L06 By default 0.1 %

L09, L36 Not determined


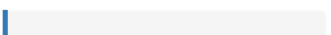
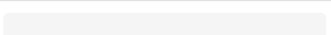
T1: 9.a. Oilseed rape MON88302: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		0	0 %
Delta Cq method (one calibration curve)		0	0 %
Digital PCR (no calibration curve)		0	0 %
No Answer		78	100 %


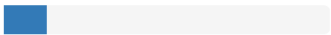
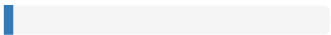
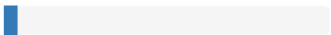
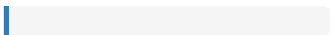
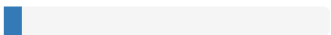
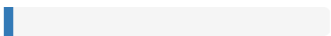
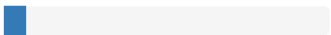
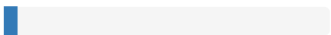
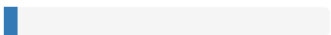
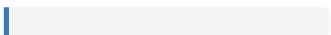
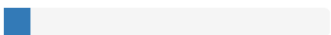
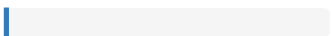
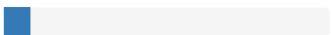
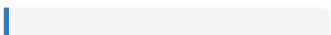
T1: 10. Why did you not analyse test item 1?

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		4	5.13 %
b) The methods are not validated in our laboratory		3	3.85 %
c) We could not obtain sufficient good quality DNA suitable for further analysis		0	0 %
d) Reference material, primers, probes, or other reagents were not available (in time)		2	2.56 %
e) We tried but our analysis failed		0	0 %
f) Other practical constraints (instrument broken, no personnel, etc.)		0	0 %
g) Other reason		0	0 %
No Answer		71	91.03 %


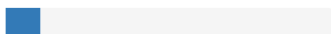
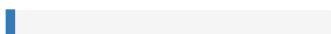
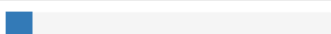
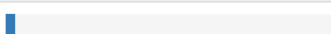
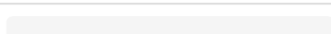
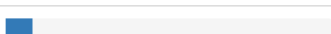
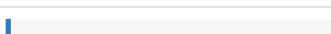
T2: Please select the option that applies and proceed with the questionnaire (you may need to wait a few seconds before all additional questions open).

		Answers	Ratio
T2 was analysed: go to Q1		77	98.72 %
T2 was not analysed: go to Q10		1	1.28 %
No Answer		0	0 %

T2: 1. Select the DNA extraction method used for T2.

		Answers	Ratio
CTAB		37	47.44 %
NucleoSpin Food		10	12.82 %
NucleoSpin Plant		2	2.56 %
GeneSpin		3	3.85 %
Promega Wizard		1	1.28 %
DNeasy Plant		4	5.13 %
DNeasy Mericon Food		2	2.56 %
Biotecon Foodproof		5	6.41 %
SDS		3	3.85 %
Fast ID Genomic DNA		3	3.85 %
Maxwell 16 Plant DNA		1	1.28 %
Maxwell 16 Food, Feed, Seed		6	7.69 %
Generon Ion Force		1	1.28 %
Other		6	7.69 %
No Answer		1	1.28 %

T2: 2. Select any additional DNA purification method used for T2.

		Answers	Ratio
No additional clean-up		53	67.95 %
Additional ethanol precipitation		8	10.26 %
Eurofins DNAExtractor cleaning column		2	2.56 %
Promega Wizard DNA clean-up resin		6	7.69 %
Qiagen QIAQuick		2	2.56 %
Qiagen Genomic-Tip 20/G		0	0 %
Other method (no need to specify)		6	7.69 %
No Answer		1	1.28 %

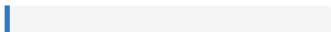
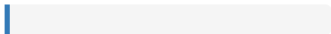
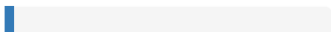

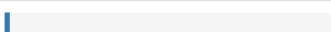
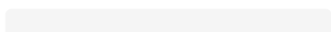
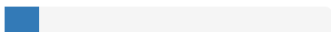


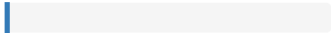
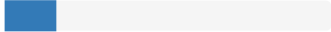
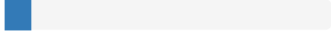
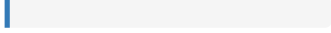
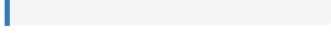
T2: 3. Indicate the number of replicate DNA extractions used to obtain the results.

		Answers	Ratio
1		0	0 %
2		54	69.23 %
3		5	6.41 %
4		10	12.82 %
5		3	3.85 %
6		4	5.13 %
>6		1	1.28 %
No Answer		1	1.28 %


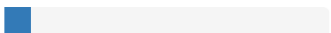
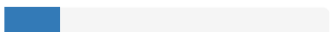
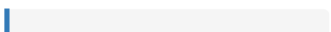
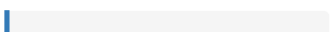
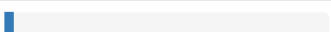
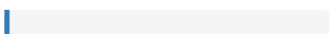
T2: 4. Select the approach used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		4	5.13 %
We run two dilutions and verify if the delta Cq or GM% are as expected		28	35.9 %
We run three or four dilutions and verify if the delta Cq or GM% are as expected		9	11.54 %
We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq of undiluted extract, compare this to the measured Cq		16	20.51 %
We add an internal positive control to the reactions and check the Cq		10	12.82 %
We verify that the amplification curves look normal		20	25.64 %
We check that the optical density ratios (OD260/280, 260/230) are acceptable		38	48.72 %
Other		2	2.56 %
No Answer		1	1.28 %

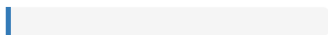
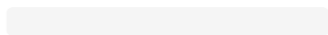
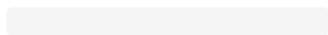

T2: 5. Which GM events were detected and quantified in T2?

		Answers	Ratio
None		1	1.28 %
44406 was detected and quantified (Q7a-7f will open)		1	1.28 %
MON87701 was detected and quantified (Q8a-8f will open)		2	2.56 %
MON89788 was detected and quantified (Q9a-9f will open)		67	85.9 %
44406 was detected, but not quantified		1	1.28 %
MON87701 was detected, but not quantified		0	0 %
MON89788 was detected, but not quantified		8	10.26 %
44406 was tested but found absent		55	70.51 %
MON87701 was tested but found absent		62	79.49 %
MON89788 was tested but found absent		1	1.28 %
44406 was not tested		12	15.38 %
MON87701 was not tested		6	7.69 %
MON89788 was not tested		1	1.28 %
No Answer		1	1.28 %

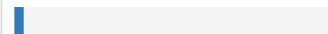
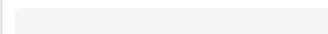
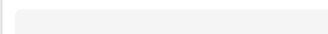

T2: 6. If applicable, why did you not test or quantify all GM events in T2?

		Answers	Ratio
a) Not applicable, all GM events listed were tested and all those detected were quantified		57	73.08 %
b) The event-specific detection method is not validated in our laboratory		6	7.69 %
c) Reference material, primers, probes, or other reagents were not available (in time)		13	16.67 %
d) The result obtained was below the LOD /LOQ		1	1.28 %
e) Practical constraints (instrument broken, no personnel, etc.)		1	1.28 %
f) Other reason		2	2.56 %
No Answer		1	1.28 %


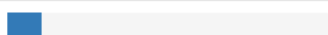
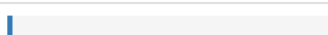

T2: 7.a. Soybean 44406: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		1	1.28 %
Delta Cq method (one calibration curve)		0	0 %
Digital PCR (no calibration curve)		0	0 %
No Answer		77	98.72 %



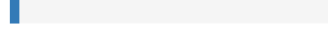


T2: 8.a. Soybean MON87701: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		2	2.56 %
Delta Cq method (one calibration curve)		0	0 %
Digital PCR (no calibration curve)		0	0 %
No Answer		76	97.44 %


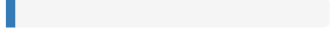
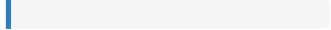
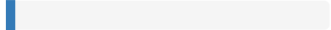
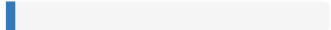
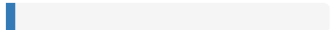
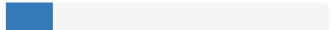
T2: 9.a. Soybean MON89788: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		60	76.92 %
Delta Cq method (one calibration curve)		8	10.26 %
Digital PCR (no calibration curve)		1	1.28 %
No Answer		11	14.1 %

T2: 9.b. Select the calibrant used for the MON89788 standard curve.

		Answers	Ratio
CRM from AOCS, certified for GM presence (assuming 100% purity)		65	83.33 %
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction		2	2.56 %
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		0	0 %
No calibrant used, digital PCR done		0	0 %
No Answer		11	14.1 %

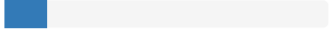
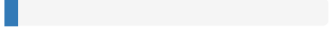
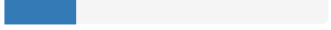
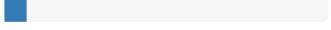
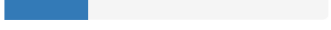
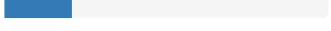
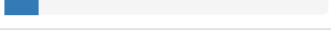
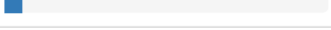
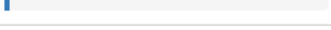
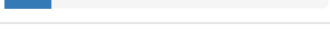
T2: 9.c. Select the endogenous target(s) used for relative quantification of MON89788 (in brackets: the event(s) for which this target was originally validated or the literature reference).

		Answers	Ratio
Soybean lec 74 bp (40-3-2, MON89788, MON87701, 44406, 356043, 305423, etc.)		58	74.36 %
Soybean lec 81 bp (Pauli et al., 2001)		2	2.56 %
Soybean lec 102 bp (A5547, FG72)		1	1.28 %
Soybean lec 105 bp (A2704)		2	2.56 %
Soybean lec 118 bp (Shindo et al., 2002)		2	2.56 %
Other, please specify below		2	2.56 %
No Answer		11	14.1 %

Specify the reference target(s) used (if different from above):

- L14 Terry C F, Harris N. Event-specific detection of Roundup Ready Soya using two different real time PCR detection chemistries. Eur. Food Res. Technol. (2001) 213:425-431.
 L67 Lektin primers Sltm1, Sltm2 og Sltmp. 80 bp. Iso method, Vaitilingom et al.

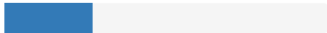
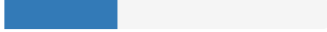
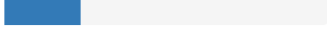

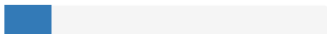
T2: 9.d. What was the amount of DNA (ng) used per PCR for MON89788? Choose the concentration that is closest to what you used. If applicable, select multiple concentrations (e.g. if several dilutions were tested) but only those of which the result was used to determine the reported GM %.

		Answers	Ratio
DNA concentration not determined		10	12.82 %
250 ng		3	3.85 %
200 ng		17	21.79 %
150 ng		5	6.41 %
100 ng		20	25.64 %
50 ng		16	20.51 %
25 ng		8	10.26 %
15 ng		4	5.13 %
<10 ng		1	1.28 %
No Answer		11	14.1 %

T2: 9.e. What was the LOQ (in m/m %) for the MON89788 quantification?

Value	Answers	Value	Answers
0.01	1	0.1	22
0.02	2	0.16	1
0.03	1	0.2	3
0.04	3	0.22	1
0.05	1	0.28	1
0.08	2	0.4	1
0.09	11	0.45	1
		No Answer	27

T2: 9.f. How was the LOQ for MON89788 determined (if applicable)?

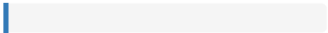
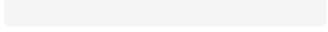
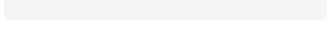
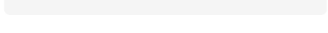




		Answers	Ratio
Determined from the qPCR analysis for the current sample		21	26.92 %
Determined during the in-house validation of the method		27	34.62 %
Taken from the EURL GMFF validation report		18	23.08 %
By another approach, please explain below		3	3.85 %
No Answer		11	14.1 %

Explanation on alternative LOQ determination (MON89788):

L06 By default 0.1 %

L09, L36 Not determined

T2: 10. Why did you not analyse test item 2?

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		1	1.28 %
b) The methods are not validated in our laboratory		0	0 %
c) We could not obtain sufficient good quality DNA suitable for further analysis		0	0 %
d) Reference material, primers, probes, or other reagents were not available (in time)		0	0 %
e) We tried but our analysis failed		0	0 %
f) Other practical constraints (instrument broken, no personnel, etc.)		0	0 %
g) Other reason		0	0 %
No Answer		77	98.72 %

Additional comments and suggestions

L01: As OCL, for resource optimisation reasons, GMFF testing is performed at qualitative level only; in routine activity in case of GM event detection samples are submitted to NLR for quantification.

L14: "For T1 the two found canola events were not verified in house. Testing for canola is not standard procedure at the NVWA. The focus for the NVWA is on foodsamples.

For T2 MON89788 is not yet in house verified.

L17: As not all CRMs, primers and probes were available, we performed a screening step. The absence of some events was inferred from the presence/absence of regulatory sequences.

L18: We had some problems with DNA extraction. First isolates, both rapeseed and soybean, had bad 260/280 ratio and quality of DNA amount was poor, that's why we repeat isolation twice.

L21: Can we request for certificate of participation as well as results of the PT for documentation purposes?

L28: Characterized control material of MON87701 is required urgently. Certified reference material in the range of 0.9% is ideal, but material characterized by comparative testing is an acceptable alternative.

Nice questionnaire.

L30: 73496 and 44406 events are not our analysing scope. In these events we are not validated so we did not test them. (73496 for T1 Item, 44406 for T2 Item).

L32: For DNA isolation is used Qiagen DNeasy Mericon Food kit.

L34: Quantification of 73496: the QN method verification is not yet completed. This analysis is performed out of accreditation.

L37: We only conducted a qualitative test, because the Reference Materials were not available.

L41: Sample T1 was tested and found absent for MON 88302 and not for MON 83302.

L46: It was very difficult to isolate DNA from RT73 reference material with promega Wizard method. We had inhibitor in the beginning. So, we had to use additional clean-up step using Epigenetics DNA clean and concentrator 25 kit.

L61: Quantitative Analysis on MON89788 soybean (only) was done by another Lab, which is quantifying all our GM soy samples as subcontractor routinely. This Lab is accredited under ISO 17025 for this measurements (Landesamt fuer Verbraucherschutz Sachsen-Anhalt, Prof. D. Maede). Our Lab is accredited only for qualitative GM soy analysis.

L63: We tried to verify & use the JRC method to quantify 73496 but although the extracted DNA passed the inhibition check with CruA, the 73496 standard curve was far outwith the requirements (i.e. slope & delta CT).

We extracted 2 standard curves and two samples for GT73 and quantified both samples with both standard curves to give a total of 4 quantification results which were averaged."

L64: The detection of the event GT73 in sample T1 was performed indirectly by detection of screening sequences PFMV and CTP2CPA EPS PS.

L67: T1 was purified four times in replicates because the different sets of purification gave quite different results in quantification (range 0.11 to 1. 7 for DP73496 and 0.11 to 0.60 for GT73). It must somehow link to the DNA purification because the two replicates in the same purification did correspond very nicely. The PCR results from the different purification sets did also correspond nicely in 2 or 3 different PCR runs. Quite strange!

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.

As the results of proficiency tests for the analysis of GMOs are generally log-normally distributed (skewed), the participant's results (NRLs only) were first \log_{10} -transformed, then the robust mean^(12,13) ($\mu_{R,log}$) was calculated on these \log_{10} -transformed data^(20,21).

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

$$z_i = (\log_{10} x_i - \mu_{R,log}) / \hat{\sigma} \quad (A3.1)$$

where: $\mu_{R,log}$ is the robust mean calculated on the \log_{10} -transformed results

$\hat{\sigma}$ is the agreed standard deviation for comparative testing, set by the Advisory Board at 0.2 for T1 and 0.15 for T2 (on the log scale).

Note that calculating the robust mean of the \log_{10} -transformed results ($\mu_{R,log}$) is not the same as taking the \log_{10} of the robust mean calculated on the raw or not-transformed data and reported throughout this report (μ_R). As a consequence, results which are identical to the robust mean on the normal scale may receive a z-score (calculated with $\mu_{R,log}$) that is deviating from 0.0. Likewise, results near the acceptance boundaries may lead to a z-score that is either within or outside the satisfactory z-score range. These are consequences of the approach used to ensure that the data distribution, which is often non-symmetrical on the normal scale, approaches a normal distribution.

Annex 4: Participants' quantitative results and z-scores

The z-scores of all laboratories are reported in Tables A4.1-A4.3. For consistency, all decimal numbers were rounded to one (z-scores) or two digits (data from participants). "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 on the \log_{10} -transformed data (see Annex 3).

Table A4.1. Results and z-scores of NRL/882 participants in comparative test ILC-EURL-GMFF-CT-02/16.

(- = not available)

Laboratory Code	Test Item 1						Test Item 2		
	73496 Oilseed Rape ($\mu_R = 0.50$ m/m %)			GT73 Oilseed Rape ($\mu_R = 0.30$ m/m %)			MON89788 Soybean ($\mu_R = 0.82$ m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score
L02	1.38	0.39	2.4	0.51	0.14	1.3	0.89	0.25	0.3
L03	0.22	0.02	-1.6	0.16	0.02	-1.3	0.64	0.09	-0.7
L05	-	-	-	0.19	0.08	-0.9	0.71	0.21	-0.4
L06	0.52	0.15	0.2	0.38	0.11	0.6	0.78	0.22	-0.1
L09	0.40	-	-0.3	0.20	-	-0.8	0.81	0.18	0.0
L10	-	-	-	0.11	0.05	-2.1	0.70	0.22	-0.4
L13	0.51	0.09	0.2	0.36	0.16	0.5	0.93	0.34	0.4
L15	-	-	-	0.45	0.14	1.0	0.79	0.25	-0.1
L17	-	-	-	0.39	0.44	0.7	2.37	1.47	3.1
L18	-	-	-	0.35	0.08	0.4	0.50	0.10	-1.4
L19	-	-	-	-	-	-	0.71	0.20	-0.4
L24	0.78	0.18	1.1	0.27	0.07	-0.1	0.56	0.16	-1.1
L25	-	-	-	0.07	0.02	-3.0	0.60	0.19	-0.9
L27	0.57	0.10	0.5	0.39	0.09	0.7	0.79	0.08	-0.1
L29	0.19	-	-1.9	0.14	-	-1.5	1.06	-	0.8
L34	0.51	0.11	0.2	0.31	0.11	0.2	0.94	0.19	0.4
L36	0.45	0.26	-0.1	0.21	0.06	-0.7	0.76	0.19	-0.2
L38	0.69	0.10	0.9	0.22	0.08	-0.6	0.89	0.25	0.3
L39	0.29	0.10	-1.0	0.26	0.12	-0.2	1.07	0.07	0.8
L41	-	-	-	0.10	0.01	-2.3	0.30	0.09	-2.9
L42	0.07	0.02	-4.1	0.00	0.00	^a	0.01	0.01	-12.7
L44	0.33	0.14	-0.7	0.27	0.18	-0.1	0.60	0.38	-0.9
L46	0.52	0.16	0.2	0.21	0.06	-0.7	0.64	0.19	-0.7
L48	0.51	0.11	0.2	0.18	0.04	-1.0	0.82	0.45	0.1
L55	0.27	0.04	-1.2	0.22	0.06	-0.6	0.84	0.29	0.1
L62	0.35	-	-0.6	0.18	-	-1.0	1.07	0.40	0.8
L67	0.50	0.10	0.2	0.12	0.04	-1.9	0.85	0.32	0.2
L68	0.84	0.25	1.3	0.37	0.11	0.6	1.14	0.34	1.0
L69	1.21	0.51	2.1	0.87	0.53	2.4	0.96	0.38	0.5
L71	-	-	-	0.35	-	0.4	0.68	-	-0.5
L78	0.31	0.14	-0.9	0.18	0.08	-1.0	0.63	0.18	-0.7

^a The laboratory reported to have quantified the GM event and to have found the result shown in the table, which is unsatisfactory.

Table A4.2. Results and z-scores of NRL/120 participants in comparative test ILC-EURL-GMFF-CT-02/16.
(- = not available)

Laboratory Code	Test Item 1						Test Item 2		
	73496 Oilseed Rape ($\mu_R = 0.50$ m/m %)			GT73 Oilseed Rape ($\mu_R = 0.30$ m/m %)			MON89788 Soybean ($\mu_R = 0.82$ m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score
L14	-	-	-	-	-	-	0.98	-	0.6
L20	1.31	0.32	2.3	0.80	0.12	2.2	1.13	0.18	1.0
L28	0.34	0.04	-0.7	0.34	0.09	0.4	1.09	0.09	0.9
L31	0.29	0.07	-1.0	3.28	0.35	5.3	1.02	0.08	0.7
L33	0.92	0.20	1.5	-	-	-	-	-	-
L35	0.53	0.04	0.3	0.24	0.06	-0.4	0.65	0.13	-0.6
L45	0.20	-	-1.8	0.20	-	-0.8	0.90	-	0.3
L49	0.59	0.18	0.5	0.46	0.14	1.0	0.82	0.30	0.1
L50	0.40	0.12	-0.3	0.95	0.29	2.6	0.80	0.24	0.0
L52	0.67	0.07	0.8	0.45	0.04	1.0	1.06	0.11	0.8
L59	0.46	0.09	0.0	0.36	0.04	0.5	0.30	0.03	-2.9
L63	-	-	-	0.31	0.04	0.2	-	-	-
L72	0.29	0.07	-1.0	3.28	0.35	5.3	0.69	0.10	-0.4
L73	0.76	0.17	1.1	0.44	0.05	0.9	0.97	0.12	0.5
L79	0.38	0.01	-0.4	0.30	0.02	0.1	0.84	0.09	0.1
L80	0.71	0.30	0.9	0.22	0.10	-0.6	0.82	0.36	0.1
L82	0.43	0.06	-0.2	0.26	0.04	-0.2	0.93	0.09	0.4

Table A4.3. Results and z-scores of non-NRL participants in comparative test ILC-EURL-GMFF-CT-02/16.
(- = not available)

Laboratory Code	Test Item 1						Test Item 2		
	73496 Oilseed Rape ($\mu_R = 0.50$ m/m %)			GT73 Oilseed Rape ($\mu_R = 0.30$ m/m %)			MON89788 Soybean ($\mu_R = 0.82$ m/m %)		
	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score	Result (m/m %)	Uncertainty (m/m %)	z-score
L04	<0.01	-	^a	0.20	0.08	-0.8	1.00	0.15	0.6
L07	-	-	-	0.36	-	0.5	-	-	-
L08	-	-	-	-	-	-	0.59	-	-0.9
L21	0.21	-	-1.7	0.37	-	0.6	0.47	-	-1.5
L22	-	-	-	-	-	-	0.59	-	-0.9
L26	-	-	-	0.20	0.10	-0.8	0.90	0.45	0.3
L30	-	-	-	0.07	-	-3.0	0.42	-	-1.9
L32	-	-	-	0.42	0.15	0.8	0.25	0.09	-3.4
L40	-	-	-	0.83	0.30	2.3	0.93	0.30	0.4
L43	-	-	-	3.15	0.78	5.2	1.16	0.34	1.1
L47	-	-	-	0.14	0.03	-1.5	-	-	-
L51	-	-	-	0.33	0.10	0.3	1.45	0.10	1.7
L53	0.71	0.23	0.9	-	-	-	0.61	0.00	-0.8
L56	-	-	-	0.41	0.20	0.8	0.66	0.24	-0.6
L58	-	-	-	-	-	-	2.09	-	2.8
L60	-	-	-	-	-	-	0.70	0.21	-0.4
L61	0.30	0.05	-0.9	3.93	0.23	5.7	1.04	0.34	0.7
L64	-	-	-	-	-	-	1.46	0.20	1.7
L65	0.58	0.20	0.5	0.19	0.04	-0.9	1.03	0.30	0.7
L66	1.08	0.16	1.8	0.36	0.12	0.5	1.24	0.32	1.2
L70	0.58	-	0.5	0.55	-	1.4	0.61	-	-0.8
L74	0.39	0.06	-0.4	0.26	0.09	-0.2	0.67	0.20	-0.5
L75	0.51	0.14	0.2	0.29	0.11	0.0	1.00	0.36	0.6
L77	0.08	-	-3.8	0.22	-	-0.6	0.64	-	-0.7
L81	-	-	-	0.25	-	-0.3	-	-	-
L83	1.40	-	2.4	0.51	-	1.3	0.84	-	0.1

^a The laboratory reported to have quantified the GM event and to have found the result shown in the table, which is unsatisfactory.

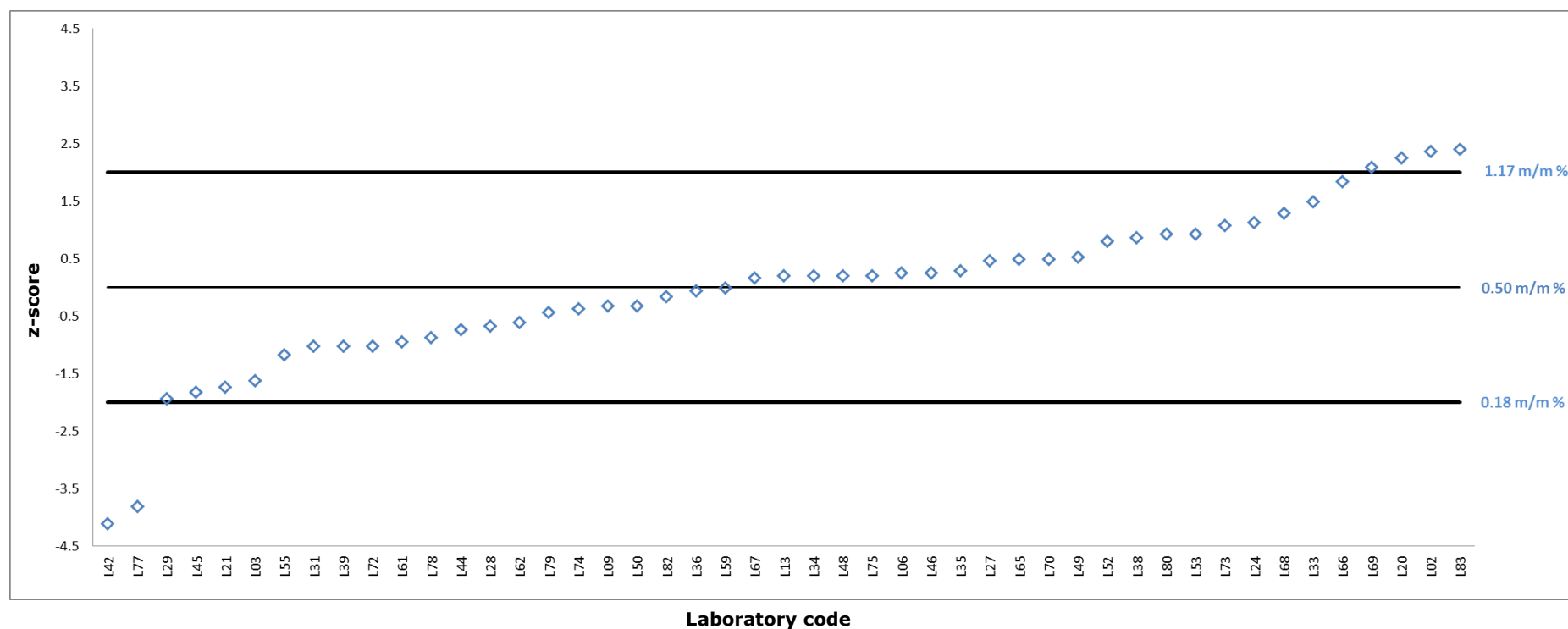


Figure A4.1. Z-scores for oilseed rape event 73496 in test item 1 on the basis of a robust mean of 0.50 m/m % (\diamond). The bold horizontal lines represent the +2 and -2 limits.

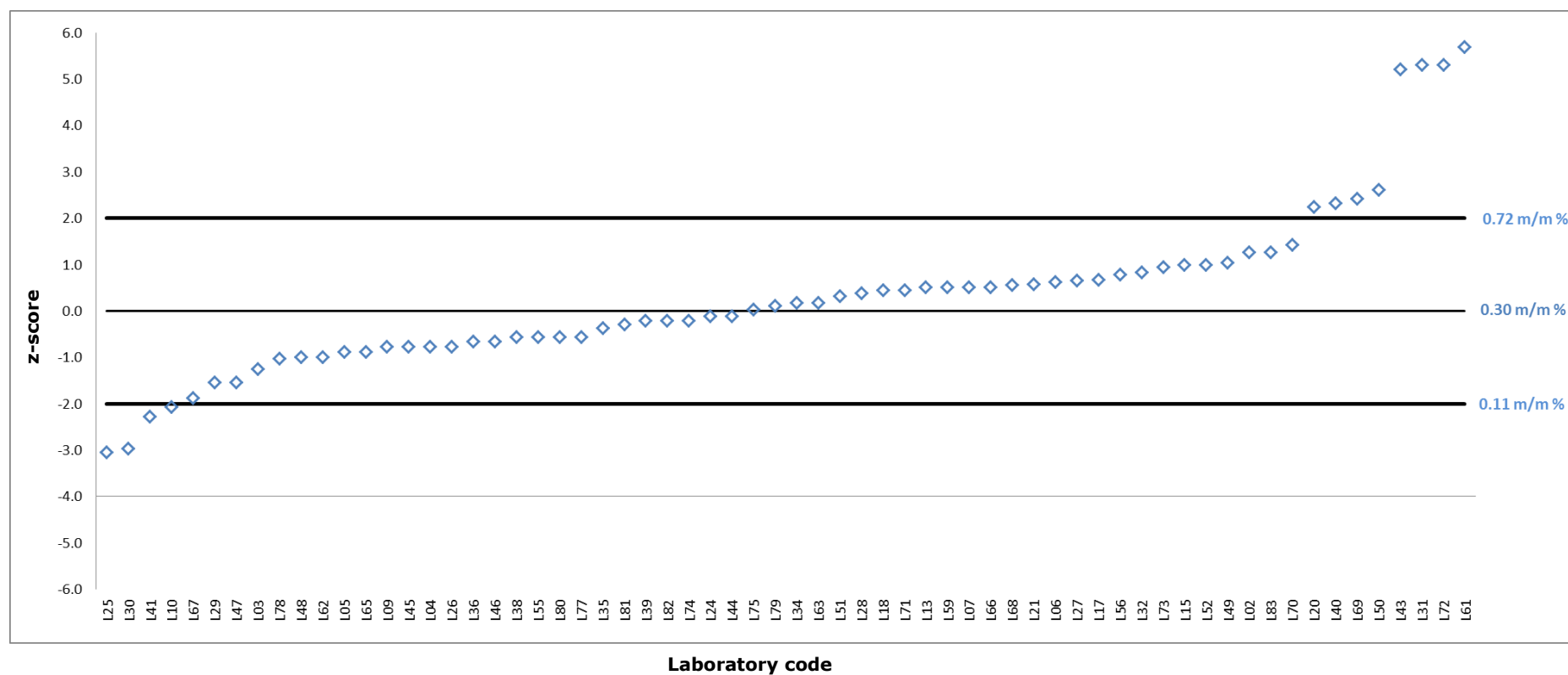


Figure A4.2. Z-scores for oilseed rape event GT73 in test item 1 on the basis of a robust mean of 0.30 m/m % (\diamond). The bold horizontal lines represent the +2 and -2 limits.

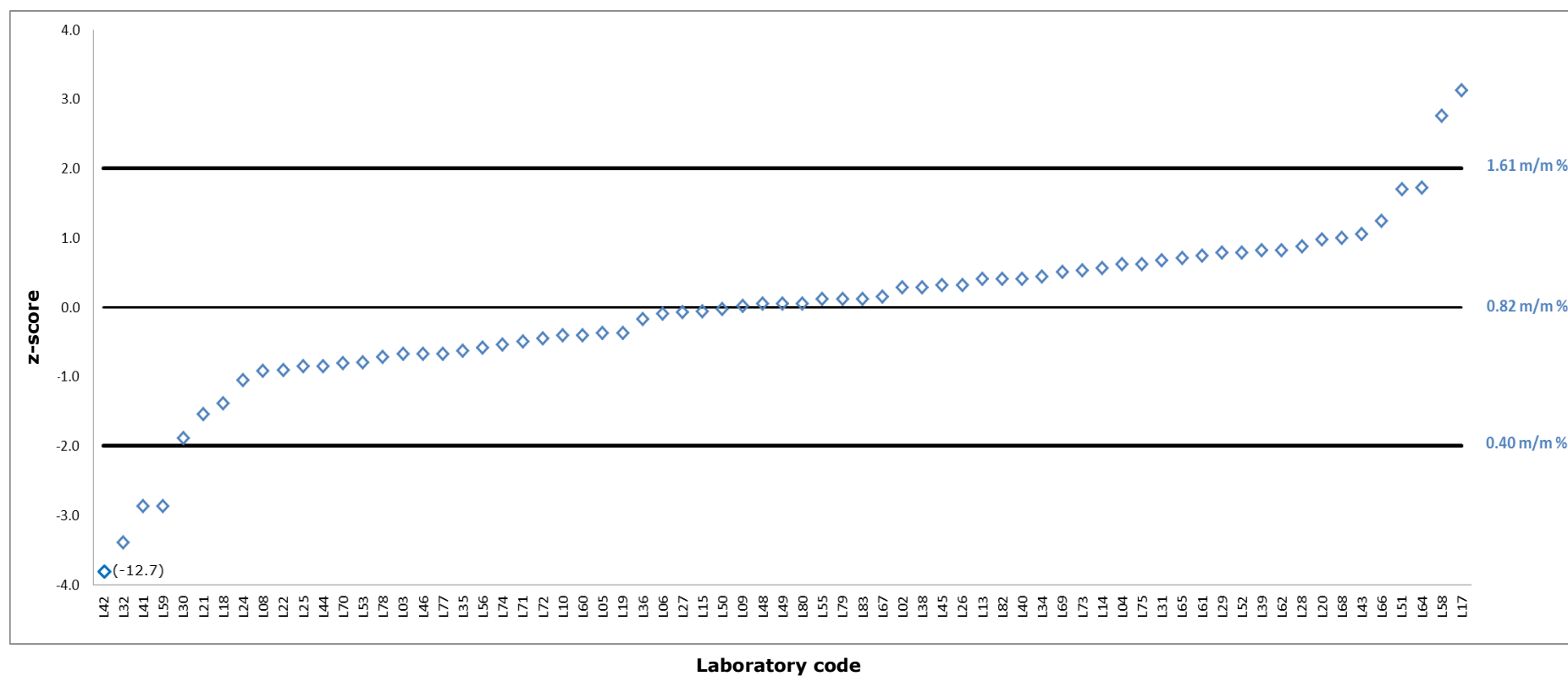


Figure A4.3. Z-scores for soybean event MON89788 in test item 2 on the basis of the assigned value of 0.82 m/m % (\diamond). The bold horizontal lines represent the +2 and -2 limits. Note that the z-score of L42 (-12.7) is not shown to scale.

Annex 5: Summary of participants' performance

The performance for detection and quantification of the three GM events in the test items provided is summarised for all participants in the Tables A5.1-A5.3; the results are shown per category of participants. "Total (un)satisfactory" is the summing up of the participants who had provided (un)acceptable qualitative or quantitative results. Unsatisfactory z-score results are shown in bold (unless the event was not analysed).

Table A5.1. Performance of NRL/882 participants in comparative test ILC-EURL-GMFF-CT-02/16. (X = identified; - = not identified; NA = event not analysed)

Laboratory Code	GM Identification			GM Quantification		
	T1		T2	T1		T2
	73496	GT73	MON89788	73496	GT73	MON89788
L02	X	X	X	2.4	1.3	0.3
L03	X	X	X	-1.6	-1.3	-0.7
L05	NA	X	X	NA	-0.9	-0.4
L06	X	X	X	0.2	0.6	-0.1
L09	X	X	X	-0.3	-0.8	0.0
L10	X	X	X	NA	-2.1	-0.4
L11	NA	X	X	NA	NA	NA
L13	X	X	X	0.2	0.5	0.4
L15	X	X	X	NA	1.0	-0.1
L17	NA	X	X	NA	0.7	3.1
L18	X	X	X	NA	0.4	-1.4
L19	Not tested		X	Not tested		-0.4
L24	X	X	X	1.1	-0.1	-1.1
L25	NA	X	X	NA	-3.0	-0.9
L27	X	X	X	0.5	0.7	-0.1
L29	X	X	X	-1.9	-1.5	0.8
L34	X	X	X	0.2	0.2	0.4
L36	X	X	X	-0.1	-0.7	-0.2
L38	X	X	X	0.9	-0.6	0.3
L39	X	X	X	-1.0	-0.2	0.8
L41	X	X	X	NA	-2.3	-2.9
L42	X	X	X	-4.1	^a	-12.7
L44	X	X	X	-0.7	-0.1	-0.9
L46	X	X	X	0.2	-0.7	-0.7
L48	X	X	X	0.2	-1.0	0.1
L55	X	X	X	-1.2	-0.6	0.1
L62	X	X	X	-0.6	-1.0	0.8
L67	X	X	X	0.2	-1.9	0.2
L68	X	X	X	1.3	0.6	1.0
L69	X	X	X	2.1	2.4	0.5
L71	X	X	X	NA	0.4	-0.5
L78	X	X	X	-0.9	-1.0	-0.7
Total satisfactory	27	31	32	19	25	28
Total unsatisfactory	0	0	0	3	4	3
Event not analysed	4	0	0	9	2	1
Sample not analysed	1	1	0	1	1	0

Table A5.2. Performance of NRL/120 participants in comparative test ILC-EURL-GMFF-CT-02/16.
(X = identified; - = not identified; NA = event not analysed)

Laboratory Code	GM Identification			GM Quantification		
	T1		T2	T1		T2
	73496	GT73	MON89788	73496	GT73	MON89788
L14	X	X	X	NA	NA	0.6
L20	X ^a	X ^a	X ^a	2.3	2.2	1.0
L28	X	X	X	-0.7	0.4	0.9
L31	X	X	X	-1.0	5.3	0.7
L33	X	X	X	1.5	NA	NA
L35	X	X	X	0.3	-0.4	-0.6
L45	X	X	X	-1.8	-0.8	0.3
L49	X	X	X	0.5	1.0	0.1
L50	X	X	X	-0.3	2.6	0.0
L52	X	X	X	0.8	1.0	0.8
L59	X	X	X	0.0	0.5	-2.9
L63	X	X	Not tested	NA	0.2	Not tested
L72	X	X	X	-1.0	5.3	-0.4
L73	X	X	X	1.1	0.9	0.5
L79	X	X	X	-0.4	0.1	0.1
L80	X	X	X	0.9	-0.6	0.1
L82	X	X	X	-0.2	-0.2	0.4
Total satisfactory	17	17	16	14	11	14
Total unsatisfactory	0	0	0	1	4	1
Event not analysed	0	0	0	2	2	1
Sample not analysed	0	0	1	0	0	1

^a GM identification result was inferred from the lack of quantification result reported (questionnaire not returned).

Table A5.3. Performance of non-NRL participants in comparative test ILC-EURL-GMFF-CT-02/16. (X = identified; - = not identified; NA = event not analysed)

Laboratory Code	GM Identification			GM Quantification		
	T1		T2	T1		T2
	73496	GT73	MON89788	73496	GT73	MON89788
L01	NA	X	X	NA	NA	NA
L04	-	X	X	^b	-0.8	0.6
L07	NA	X	X	NA	0.5	NA
L08	Not tested		X	Not tested		-0.9
L12	Not tested		X	Not tested		NA
L16	Not tested		NA	Not tested		NA
L21	X	X	X	-1.7	0.6	-1.5
L22	Not tested		X	Not tested		-0.9
L23	Not tested		X ^a	Not tested		NA
L26	NA	X	X	NA	-0.8	0.3
L30	NA	X	X	NA	-3.0	-1.9
L32	NA	X	X	NA	0.8	-3.4
L37	X	X	X	NA	NA	NA
L40	X	X	X	NA	2.3	0.4
L43	NA	X ^a	X ^a	NA	5.2	1.1
L47	NA	X	X	NA	-1.5	NA
L51	NA	X	X	NA	0.3	1.7
L53	X	NA	X	0.9	NA	-0.8
L56	NA	X	X	NA	0.8	-0.6
L58	X	-	X	NA	NA	2.8
L60	Not tested		X	Not tested		-0.4
L61	X	X	X	-0.9	5.7	0.7
L64	NA	X	X	NA	NA	1.7
L65	X	X	X	0.5	-0.9	0.7
L66	X	X	X	1.8	0.5	1.2
L70	X	X	X	0.5	1.4	-0.8
L74	X	X	X	-0.4	-0.2	-0.5
L75	X	X	X	0.2	0.0	0.6
L76	Not tested		-	Not tested		NA
L77	X	X	X	-3.8	-0.6	-0.7
L81	NA	X	X	NA	-0.3	NA
L83	X	X	X	2.4	1.3	0.1
Total satisfactory	13	23	30	8	16	21
Total unsatisfactory	1	1	1	2	4	2
Event not analysed	11	1	1	15	5	9
Sample not analysed	7	7	0	7	7	0

^a GM identification result was inferred from the lack of quantification result reported (questionnaire not returned).

^b The laboratory reported a value of <0.01 m/m %, which was not attributed a z-score, but is unsatisfactory.

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