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EURL GMFF Proficiency Test Report CT 02/17

Determination of GM Soybean 40-3-2 in Chicken Feed and Soybean Flour

Broothaerts, W., Beaz Hidalgo, R., Corbisier, P., Cordeiro, F., Dimitrievska, B., Emteborg H., Maretti, M., Robouch, P., Emons, H.

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268-PT Accredited by the
Belgian Accreditation Body (BELAC)

Quality assurance

The European Union Reference Laboratory for GM Food and Feed (EURL GMFF), hosted by the Joint Research Centre (JRC-Ispra and JRC-Geel), is ISO/IEC 17025:2005 accredited (certificate number: Belac 268 TEST, flexible scope for DNA extraction, DNA identification and real-time PCR) and ISO/IEC 17043:2010 accredited (certificate number: Belac 268 PT, proficiency test provider).

Confidentiality statement

The procedures used for the organisation of PTs are accredited according to ISO 17043:2010 and guarantee that the identity of the participants and the information provided by them is treated as confidential. The participants in this PT received a unique laboratory code that is used throughout this report. Laboratory codes of the National Reference Laboratories appointed in line with Regulation (EC) No 882/2004 will be disclosed to DG SANTE for (long-term) performance assessment.

List of abbreviations

CT	Comparative test (here referred to as PT, proficiency test)
EURL	European Union Reference Laboratory
GMFF	Genetically modified food and feed
kbp	(kilo) thousand base pairs
LOQ	Limit of quantification
m/m %	Mass fraction or mass per mass percentage
NRL	National Reference Laboratory
OCL	Official Control Laboratory
qPCR	Quantitative (real-time) Polymerase Chain Reaction
SD	Standard deviation
σ_{pt}	Standard deviation for proficiency assessment
U/u	Expanded/standard measurement uncertainty
z and ζ	Performance scores used to assess the measurement capability of a laboratory

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Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised a proficiency test (PT) 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: test item 1 (T1) was composed of ground chicken feed spiked with a mixture of GM soybean event MON-Ø4Ø32-6 (40-3-2) and non-GM soybean, and test item 2 (T2) was a soybean flour containing the same GM soybean event 40-3-2. Participants were required to screen T1 and T2 for the presence of three GM soybean events, and to quantify the event that was present with the highest GM mass fraction. The results had to be reported in GM mass fraction (mass/mass %).

Eighty-six participants from 39 countries participated to this PT, including 54 NRLs, of which 33 are designated in line with Regulation (EC) No 882/2004 (NRL/882) and 21 are nominated in Regulation (EU) No 120/2014 to support the EURL GMFF on method validation (NRL/120), as well as 10 other EU official control laboratories.

The qualitative results, i.e. the correct identification of the GM event, were evaluated and scored as correct or incorrect. The assigned value for the 40-3-2 soybean mass fraction in both test materials was derived as the robust mean of the data provided by the NRLs. Laboratory performance was primarily evaluated by calculating *z* scores.

The results reported indicate that all participants identified the correct GM event in both test items. All, but one NRL obtained a satisfactory performance (*z*) score for the reported 40-3-2 soybean mass fraction in both test items and the performance of one other NRL was unacceptable for T1 because they reported the 40-3-2 mass fraction as below their LOQ. Six and two NRLs obtained a questionable *z* score for T1 and T2, respectively.

Considering the results provided by the other participants (non-NRL), three and two non-NRLs obtained an unsatisfactory *z* score for T1 and T2, respectively. Another three laboratories obtained a questionable *z* score for T1 and one laboratory for T2.

The laboratories' ability to provide results close to the assigned value within their claimed measurement uncertainty was additionally evaluated by ζ scores. Twenty four and 16 laboratories had an unsatisfactory ζ scores for T1 and T2, respectively. Unsatisfactory ζ scores were mainly the consequence of an underestimated or not reported measurement uncertainty. Guidance is provided for correctly estimating the measurement uncertainty of analytical results.

A root-cause analysis will be requested from NRLs with an unsatisfactory *z* score result in this PT and will be followed-up.

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 activities for the official control 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; several of them are also NRL/882). The Member States of the European Union may also appoint other laboratories (non-NRLs) to perform 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 proficiency tests (called comparative tests or CT in the GMO legislation[(2)]) to foster the correct application of the analytical methods available for the official controls. The EURL GMFF is operating under a quality management system which is accredited according to ISO/IEC 17043[(5)] for the organisation of proficiency testing.

This report summarises the results obtained in a PT organised by the EURL GMFF in 2017 (CT 02/17). Participation in such PTs is mandatory for NRL/882, recommended for NRL/120, and open to other official control laboratories.

2 Test items

The test items used in this PT were prepared and characterised at JRC-Geel.

2.1 Test item 1

The T1 test item was prepared from base materials that were characterised before their use (Table 1). The base materials employed for the preparation of T1 were chicken feed (AVEVE, for biological agriculture according to EC 834/2007 and EC 889/2008), the ERM-BF410ak as non GM soybean powder and the ERM-BF410bp[6] containing the MON-Ø4Ø32-6 event (hereafter named 40-3-2) as spiking material (Table 1).

The chicken feed was composed, according to the label, of Bio maize kernels, Bio soybean oil-cake, Bio-sunflower oil-cake, Bio wheat, Bio barley, maize gluten, potato proteins, calcium monophosphate, calcium carbonate, Bio soybean oil and sodium chloride. The analytical composition indicated a content of 17.5 % protein, 5 % fat, 13 % ashes, 5 % cellulose, 0.33 % methionine, 0.78 % lysine, 3.7 % calcium, 0.52 % phosphorus and 0.1 % sodium. The chicken feed was milled using a cryo-grinding vibrating mill (Palla mill, KHD, Humboldt-Wedag, Köln, DE) and sieved with a 500 µm stainless steel mesh on a sieving machine equipped with an ultrasonic sieving aid (Russel Finex, London, UK). The remaining powder was mixed in a DynaMIX CM200 (WAB, Muttenz, CH) for 1 h to homogenise the distribution of the different types of seed tissues.

The residual water mass fractions for the chicken feed powder and the powders of the certified reference materials ERM-BF410ak and ERM-BF410bp were measured by volumetric Karl Fischer titration (758 KFD Titrino, Metrohm, Herisau, CH). The results showed that the powders were sufficiently dry to perform the dry mixing and did not require an additional drying step.

The particle size distribution of the powders was measured using laser diffraction (PSA, Sympatec, Clausthal-Zellerfeld, DE). It was concluded that the particle size distribution of these powders was sufficiently similar to allow subsequent preparations of mixtures.

The amount and the quality of the DNA extracted from the chicken feed powder, the non-GM soybean flour and the GM spiking material were verified by UV spectrometry, fluorometry and gel electrophoresis. A CTAB-tip 20/G method (Qiagen, Hilden, Germany) optimised for soybean was chosen with a sample intake of 200 mg because it yielded a sufficient amount of DNA of PCR-grade quality from the base materials. DNA extracted with the in-house CTAB method was tested for PCR inhibition between 40 ng/µL to 0.2 ng/µL with a lectin qPCR assay (5 µL per PCR) and did not show any inhibition (ΔCq values were very close to the theoretical ΔCq values). The PCR efficiencies ranged from 96 to 98 % with a coefficient of determination (R^2) between 0.99 and 1.00, confirming the absence of significant amounts of PCR inhibitors in the extracts.

The CTAB method yielded a sufficient amount of DNA of PCR-grade quality from both non-GM and GM base materials.

The level of fragmentation of the extracted DNA was investigated by 1.0 % agarose gel electrophoresis. A smear from ± 12 to 1 kbp could be clearly seen in the chicken feed DNA, indicating some level of fragmentation of the extracted DNA, while the DNA extracted from the soybean materials migrated as a high molecular weight band (above 12 kbp). The amount of soybean DNA that could be extracted and amplified from the chicken feed powder was determined by qPCR with a lectin assay using DNA from a soybean CRM as calibrant; this amount appeared to be rather low (<1 % of total DNA). The yield of amplifiable DNA per mg of chicken feed powder and the yield of DNA measured by PicoGreen for the soybean materials, composed of pure soybean, were taken into account to calculate the amount of chicken feed powder, non-GM soybean and GM soybean to be mixed to obtain a target value of approximately 0.8 m/m % event 40-3-2 in T1.

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

Characteristic	Chicken Feed	Non-GM Soybean Flour	40-3-2 Soybean Flour
Type of base material	Scratch grains	CRM	CRM
Origin	AVEVE (Belgium)	ERM-BF410ak	ERM-BF410bp
Grinding method	Cryo-grinding vibrating mill	Used as such	Used as such
Mixing method	DynaMIX CM200 (WAB, Muttenz, CH)		
Water content in g/kg, mean ± *U* (*k* = 2, *n* = 3)	10.6 ± 1.5	11.5 ± 0.7	15.5 ± 1.0
Particle diameter in µm, mean ± *U* (*k* = 2, *n* = 5)	111.4 ± 19.8	118.8 ± 27.2	121.3 ± 6.0
Soybean DNA yield in ng/mg[1], mean ± *U* (*k* = 2)	0.7 ± 0.1 (*n*=5)	57.9 ± 17.8 (*n*=3)	72.9 ± 1.8 (*n*=2)
Genetic elements detected with screening pre-spotted plates (Cq value)[2]	Hmg (Cq 24.6), Lec (24.7), CruA (Cq 35.7), UGP (28.1), PLD (36.5), P35S (Cq 33.3), tNOS (Cq 36.8), CTP2-EPSPS (Cq 35.7), Cry1Ab/Ac (Cq 36.0), PAT (Cq 37.6)	Lec (Cq 21.5)	Lec (Cq 21.2), p35S (Cq 21.2), tNOS (Cq 22.5), PAT (Cq 34.8)
GM soybean events detected with event-specific pre-spotted plates (Cq value)[2]	40-3-2 (Cq 34.0), MON87701 (Cq 37.4), MON89788 (Cq 36.3), Lec (Cq 24.6)	NA	40-3-2 (Cq 21.4), A5547 (Cq 41.2)
Mass used to prepare T1 (g)	1103.2	96	1.08
Nominal target GM mass fraction in T1 (m/m %)	NA	NA	0.8

[1] Results reported here for a sample intake of 200 mg with the in-house validated CTAB method + Genomic-tip 20/G purification for soybean (JRC-GEEL). The soybean DNA yield value for the chicken feed was determined by qPCR, whereas the yield from the non-GM and GM soybean materials was measured by fluorometry.
[2] A screening and GM soybean event-specific pre-spotted plate (PSP) was used for these tests.
NA: not applicable; *k*: coverage factor; *U*: expanded uncertainty.

The presence of different species and GM events in the base materials and in a pilot mixture was tested by using the screening[(7)] and GM soybean event-specific pre-spotted plates[(8)].

The presence of maize and soybean in the chicken feed powder was confirmed by the early quantification cycle obtained for the high mobility gene (*hmg*) and lectin (*lec*) assays. Late amplifications for the UDP-glucose pyrophosphorylase (*ugp*), phospholipase D (*PLD*) and cruciferin A (*cruA*) genes confirmed the respective presence of potato, sunflower and rapeseed in the chicken feed.

The chicken feed powder (labelled "bio") also contained traces of genetic markers such as p35S, tNOS, EPSPS and Cry1Ab/Ac which indicate a contamination of the chicken feed by genetically modified plant materials. The GM soybean event-specific assays confirmed the presence of traces of 3 GM soybeans, namely: 40-3-2, MON87701 and MON89788. The level of contamination was estimated to be below 0.01 %.

The final test item was prepared gravimetrically in accordance with ISO 17034:2016[(9)] as follows:

- The mass of the GM ingredient to add (40-3-2 soybean) was calculated taking into account the amount of DNA that could be extracted and amplified from the different materials (Table 1).
- The compound sample T1 was mixed in a DynaMIX CM200 for 1.5 h to improve homogeneity.
- After finalisation of the mixing step, the powders were filled manually in 20 mL brown glass vials using lyophilisation inserts manually placed in the bottle necks. Before final closure of the vials, air was evacuated in a freeze-dryer and replaced by argon. The vials were finally closed inside the freeze-dryer with the help of a hydraulic device and then sealed with blue aluminium caps to maintain the inert atmosphere and to prevent accidental opening during storage and transport.
- A total of 200 vials containing each at least 5 g of flour were then labelled with a sample number and the description "Sample T1 (chicken feed)".

- Following the inventory and the selection of vials for future analysis according to a random stratified sampling scheme, the bottles were brought to a storage room for long-term storage in the dark at 4 ± 3 °C.

Homogeneity and stability testing of T1 was performed in-house, as described in Annex 1, using an event-specific quantification method previously validated by the EURL GMFF. Material T1 was found to be homogeneous for the GM event added (p-value > 0.05), based on a 200 mg sample intake.

From the isochronous stability study, it was concluded that the test item would be sufficiently stable under ambient shipment conditions (5 % significance level). Stability was also confirmed during the whole period of the PT, between the dispatch of the test items until the deadline of reporting the results (Annex 1).

JRC-Ispra tested the T1 material and this confirmed the results obtained by JRC-Geel. The average (n = 91) mass fraction of event 40-3-2 measured in T1 was 0.69 ± 0.04 m/m % (U, k = 2), which approximated the expected nominal value, but may have been influenced by the characteristics of the different base materials.

2.2 Test item 2

The T2 test item was a new batch of a certified reference material that was not yet released on the market (Table 2). The bottles of T2 were re-labelled with a unique sample number as "Sample T2 (soybean flour)".

Homogeneity and short-term stability of T2 had been previously demonstrated as part of the certification of the CRM; stability monitoring confirmed the stability of T2 during the running time of the PT (Annex 1).

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

Characteristic	**Soybean feed**
Type of base material	CRM
Origin	ERM-BF410dp[6] containing 10.0 ± 0.6 g/kg MON-Ø4Ø32-6 soybean, produced in 2017 by JRC-Geel.

The certificate of ERM-BF410dp warns that "*a difference (at 95 % confidence level) between the total DNA content in the two powders used for the production of ERM-BF410dp was found to be significant (due to the different size of non-GM and GM seeds) and is likely to have an impact when using this CRM. Depending on the composition of the unknown sample, real-time PCR measurement results of ERM-BF410dp may differ up to 23.9 ± 1.1 % (average ± U) compared to the results of the unknown sample. This difference may depend also on the DNA extraction method selected and both effects may be additive.*" This observation was confirmed by the participants in this PT, who used the previous 40-3-2 soybean CRM batch (ERM-BF410n) for the calibration of the T2 measurements.

3 Instructions to the participants

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

Test Item 1: "Chicken feed"

- Screen for the presence of the following three GM soybean event(s): 40-3-2, 68416 and MON89788;
- Quantify the GM soybean event that is present with the highest GM mass fraction.

Test Item 2: "Soybean flour"

- Screen for the presence of the following three GM soybean event(s): 40-3-2, 68416 and MON89788;
- Quantify the GM soybean event that is present with the highest GM mass fraction.

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

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

Participants were requested:

- to use procedures for detection/quantification of the GM events that resemble as closely as possible the ones used in routine sample analysis;
- to take care in ensuring that the DNA extraction method employed is adapted to the matrix and that the quality of the DNA obtained is suitable for PCR;
- to report the quantitative results with two decimal places (e.g. 0.64 or 1.29);
- to follow 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[10,11];
- to pay attention to the correct estimation and reporting of the measurement uncertainty and coverage factor used, as the uncertainty reported would be considered in the evaluation of the results using zeta scores;
- in case of an unsatisfactory performance, to fill in a form indicating the root-cause analysis and providing evidence demonstrating the effectiveness of the corrective actions implemented (for NRLs only).

The participants were also informed that the identification information on the participants in this PT would be kept confidential, except for the NRLs that have been appointed in line with Regulation (EC) No 882/2004; their lab codes will be disclosed to DG SANTE for the purpose of an assessment of their performance.

4 Results

4.1 Participation to the PT

On 31 May 2017, 199 laboratories were informed about the upcoming PT EURL-GMFF-CT-02/17. Finally, 89 laboratories registered for it and received a random unique lab code (L01 to L89). Eighty-six laboratories from 39 countries returned results within the reporting deadline. Three non-NRLs did not submit any results, two of which (L21, L28) had not received the samples from customs, while L67 did not provide any justification for not participating.

Table 3 shows an overview of the participation to this PT.

Table 3. Communication about and participation to the PT 02/17.

Characteristic of the PT	Result
Date of PT announcement	31 May 2017
Deadline for registration	14 June 2017
Date of shipment of samples	3 July 2017
Deadline for result submission	25 August 2017
Number of laboratories informed	199
Number of registered laboratories	89
Registered laboratories that failed to submit their data	3
Number of participating laboratories	86

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

- Thirty-three NRLs designated in line with Regulation (EC) No 882/2004 (NRL/882), representing 25 EU Member States (many of them are also NRL/120). In addition, Ireland delegated its NRL/882 tasks to one of the PT participants. Estonia and Malta were not represented in this PT.
- Twenty-one NRLs nominated under Regulation (EU) No 120/2014 (NRL/120) that are not at the same time NRL under Regulation (EC) No 882/2004.
- Thirty-two laboratories that are not NRL, but are appointed by their National Authority to perform official controls. This category includes 10 EU official control laboratories (OCLs) and 22 laboratories from non-EU countries, including Serbia and Switzerland.

Among the countries, Germany was represented with 17 laboratories, Italy with 6 laboratories, and Belgium and Poland with 4 laboratories each; all other countries had between one and three participating laboratories.

Table 4. Overview of participants to CT 02/17 by country and category.

Country	Participants	NRL/882[1]	NRL/120	Non-NRL
AUSTRIA	2	2		
BELGIUM	4	3		1
BRAZIL	2			2
BULGARIA	2	1		1
CHILE	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	17	1	14	2
GREECE	1	1		
HUNGARY	2	1		1
INDIA	1			1
ITALY	6	1	2	3
LATVIA	1	1		
LEBANON	1			1
LITHUANIA	1	1		
LUXEMBOURG	1	1		
MEXICO	1			1
NETHERLANDS	2	1	1	
PHILIPPINES	1			1
POLAND	4	3	1	
PORTUGAL	1	1		
ROMANIA	2	1		1
SERBIA	3			3
SINGAPORE	1			1
SLOVAKIA	2	2		
SLOVENIA	1	1		
SPAIN	2	2		
SWEDEN	1	1		
SWITZERLAND	2			2
TURKEY	1			1
UKRAINE	3			3
UNITED KINGDOM	3	1	2	
UNITED STATES	1			1
VIETNAM	3			3
Total	**86**	**33**	**21**	**32**

[1] No NRL/882 from Estonia or Malta participated to this PT.

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 used for T1 and T2, consisting of a number of mostly multiple-choice questions. A total of 82 laboratories completed the questionnaire, including all 33 NRL/882, 20 out of 21 NRL/120 and 29 out of 32 non-NRLs (questionnaires were missing from L03, L10, L38 and L49).

Table 5 summarises the main answers received, whereas Annex 2 shows all answers. The results on GM event identification are reported in Section 4.3.

Table 5. Summary of the main answers provided in the questionnaire of CT 02/17.

Question (and Question number)	Test Item 1 – 40-3-2	Test Item 2 – 40-3-2
Test item analysed	Yes (80[1]), No (2)	Yes (82), No (0)
Reason for lack of analysis (Q1)	Matrix out of scope (1), other practical constraints (1)	-
DNA extraction method (Q2)	CTAB (41), NucleoSpin Food (10)	CTAB (40), NucleoSpin Food (11)
Additional DNA purification method (Q3)	None (51), Ethanol (9)	None (52), Ethanol (9)
Number of replicates (Q4)	2 (52), 4 (10)	2 (56), 4 (10)
Approach to test for PCR inhibition (Q5)	OD ratios (40), delta Cq or GM % between two dilutions (31)	OD ratios (36), delta Cq or GM % between two dilutions (32)
Reason for not testing all events (Q9)	Not applicable (45), below the LOQ (18)	Not applicable (61), reagents not available (10)
Approach used (Q6a)	Standard curves (68), delta Cq (9)	Standard curves (71), delta Cq (9)
Calibrant used (Q6b)	CRM JRC-Geel in g/kg (71), other RM in copies (4)	CRM JRC-Geel in g/kg (74), other RM in copies (4)
Taxon-specific endogenous gene (Q6c)	*lec*-74 bp (65), *lec*-81 or 118 bp (each 5)	*lec*-74 bp (63), *lec*-118 bp (6)
Unit of measurement and data expression (Q6d)	Mass (59), copies=mass CRM (13)	Mass (61), copies=mass CRM (14)
Amount of DNA (Q6e)	200 ng (30), 100 ng (18)	200 ng (30), 100 ng (21)
LOQ (Q6f)	0.1 (37), <0.1 (34)	0.1 (39), <0.1 (39)
LOQ determination (Q6g)	In-house validation (42), current analysis (20)	In-house validation (43), current analysis (21)
Uncertainty determination (Q6h)	Precision of replicates (32), in-house validation (27)	Precision of replicates (32), in-house validation (30)

[1] The numbers shown refer to the number of laboratories that reported the answer. The answers that were reported with the two largest frequencies are mentioned.

One NRL/882 (L73) reported that T1 was out of the scope of the laboratory, and one non-NRL (L43) reported that the T1 matrix was not analysed because of practical constraints.

The evaluation of the answers shows 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. No additional purification methods were generally applied. The majority of laboratories analysed two replicate DNA extracts. Most laboratories checked the quality of the DNA extracts by verifying the OD ratios, and/or running two dilutions; a minority of laboratories performed a PCR inhibition run on 3 or 4 DNA dilutions with a reference gene.

For the quantitative analysis, the most common approach used was based on two standard curves, however, 9 laboratories applied the delta Cq approach. One laboratory (L50) mentioned the use of digital PCR for 40-3-2 soybean quantification in T1 and T2. The available CRMs from the JRC were used by most laboratories, but 5 laboratories used a non-certified reference material (RM) where values were expressed in GM copy number ratio (4) or GM mass fraction (1). *Lec* was used as taxon-specific reference gene by all laboratories for soybean (mostly the 74 bp version). The majority of laboratories performed their measurements in the same unit as the certified value of the calibrant used (g/kg) and no conversion factor was applied. The LOQ reported was either taken from in-house validation of the method or determined from the analysis results for this CT. In most cases a LOQ of 0.1 m/m % or lower was reported. The measurement uncertainty was either derived from the standard deviation of the measurement replicates or from the intermediate precision determined in the frame of the single-laboratory validation study.

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.

Table 6. Summary of GM event identification results of the participants as reported in the questionnaire or (in brackets) inferred from the quantitative result reported.

Laboratories	Test Item	GM Event	Present	Absent	Not Tested	Sample Not Analysed
NRL/882 and NRL/120	T1	40-3-2	52 (+1)	0	0	1
		68416	0	51	1	
		MON89788	32	19	1	
	T2	40-3-2	53 (+1)	0	0	0
		68416	0	52	1	
		MON89788	5	47	1	
Non-NRLs	T1	40-3-2	28 (+2)	0	0	1
		68416	1	14	13	
		MON89788	16	7	5	
	T2	40-3-2	29 (+2)	0	0	0
		68416	1	14	14	
		MON89788	4	21	4	

All 53 NRLs who had tested T1 identified the 40-3-2 event in T1. The 68416 soybean event was found absent in T1, whereas MON89788 soybean was detected by 32 NRLs in T1. Seven NRLs also reported to have quantified MON89788 in T1 and while most laboratories reported the GM mass fraction as being below the LOQ, two laboratories reported a GM mass fraction of 0.02 and 0.025 m/m %. The presence of traces of MON89788 soybean in the chicken feed was indeed confirmed by JRC-Geel (see Table 1).

For T2, all 54 NRLs identified the 40-3-2 event and found 68416 absent. MON89788 soybean was detected by 5 NRLs, but quantified as below the LOQ (note that the presence of MON89788 in T2 was not confirmed by JRC-Geel). The results show that EU NRLs are able to correctly identify the 40-3-2 soybean event in both a compound feed matrix and in soybean flour.

The results of all non-NRLs were also satisfactory for event 40-3-2 soybean, however, a larger proportion of laboratories did not test the event MON89788 and, particularly, 68416.

The performance of all laboratories for qualitative identification of the correct GM events is summarised in Annex 3.

4.4 GM event quantification

4.4.1 Number of participants reporting a quantitative result

Table 7 presents the number of laboratories having submitted quantitative data for the GM event present in the test items. A large majority of participating laboratories reported a quantitative result for 40-3-2 soybean in T1 (93 %) and T2 (97 %). Among the NRLs, one NRL/120 (L88) provided a result for 40-3-2 soybean in T2, but not for T1, reporting that the mass fraction of the 40-3-2 event was below the LOQ in T1. All NRL/882 participants quantified the event in both test items, except L73, for which the T1 matrix was out of their scope.

Expanded measurement uncertainties were reported by the NRLs for all measurement results, with the coverage factor reported for 88 and 87 % of the results for T1 and T2, respectively (Table 7). Although the results show that most control laboratories understand the principle that analytical results should be reported with an expanded uncertainty, when asked, it is unclear why some laboratories did not report the coverage factor (*k)* used to convert the standard uncertainty into an expanded uncertainty that corresponds to a 95 % level of confidence.

Table 7. Number of laboratories reporting a quantitative GM event-specific result.

Quantitative Results Reported	Test Item 1 – 40-3-2 Soybean			Test Item 2 – 40-3-2 Soybean		
	NRL/882	NRL/120	Non-NRL	NRL/882	NRL/120	Non-NRL
Total participants	33	21	32	33	21	32
Quantitative result	32[a]	20[b]	28[c]	33	21	29[c]
Measurement uncertainty	32	20	19	33	21	20
Coverage factor	28	18	17	28	19	18

[a] L73 (NRL/882) did not analyse T1 as the matrix is out of the scope of the laboratory.
[b] L88 (NRL/120) did not provide a quantitative result for 40-3-2 soybean in T1.
[c] L10, L11 and L71 (non-NRLs) have not reported a quantitative result for T1 and T2; L43 has not analysed T1.

4.4.2 Assigned values

The assigned value (x_{pt}) for the mass fraction of event 40-3-2 soybean in T1 and T2 was based on the consensus value of the data from a pre-selected part of participants in this PT, calculated using robust statistics[12,13]. This statistical approach minimises the influence of outlying values. The data taken into account for the calculation of the robust means were those from the NRLs (NRL/882 and NRL/120) only. The data from non-NRLs were excluded because of the heterogeneity of this group with regard to experience in GMO analysis.

The results of proficiency tests for the analysis of GMOs are often log-normally distributed (skewed)[14,15]. This was not the case for the results of this PT; however, for consistency with previous PTs, the same approach was followed as in previous rounds. The results reported by the NRLs were first $\log_{10}$-transformed, and the robust mean ($x_{pt\text{-}log}$) and corresponding robust standard deviation (s^*) were calculated. The standard measurement uncertainty [$u(x_{pt\text{-}log})$] of the assigned value is assumed to include the effects of uncertainty due to inhomogeneity and instability; it is estimated according to ISO 13528:2015 (section 7.7.3)[16], as follows:

$$u(x_{pt-log}) = 1.25 \frac{s^*}{\sqrt{N}} \qquad (2)$$

where: s^* = robust standard deviation of the results expressed in m/m % (log scale);
N = number of results used for the calculation (from NRLs only).

A coverage factor (k) of 2 was used to calculate the expanded uncertainty (U) corresponding to a 95 % level of confidence[17].

The assigned values and associated uncertainties for 40-3-2 soybean in both test items are reported in Table 8. The standard deviation for proficiency assessment ($\sigma_{pt\text{-}log}$) was set to 0.10 (on the log scale) for both test items, based on reasonable performance expectations and experience from previous PTs.

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

Variable	40-3-2 Soybean in T1	40-3-2 Soybean in T2
Assigned value derived as	Robust mean of $\log_{10}$-transformed data	Robust mean of $\log_{10}$-transformed data
Number of data points (NRLs)	52	54
Assigned Value ($x_{pt\text{-}log}$)	**-0.0973**[a]	**-0.1186**[b]
Standard uncertainty [$u(x_{pt\text{-}log})$]	0.0207	0.0122
Standard deviation for proficiency assessment ($\sigma_{pt\text{-}log}$)	0.10	0.10

[a] The assigned value for the mass fraction of 40-3-2 soybean in T1 corresponds to an approximate GM % in the raw domain of 0.80 m/m %.
[b] The assigned value for the mass fraction of 40-3-2 soybean in T2 corresponds to an approximate GM % in the raw domain of 0.76 m/m %.

The robust mean of the 40-3-2 soybean mass fraction reported for T2 was 24 % lower (on the raw scale) than the certified value of this new batch of CRM[6], which was released in October 2017 (i.e. after this PT). This observation was already noticed during CRM production and a note on this is included in the CRM certificate (see Section 2.2 in this PT

report). Laboratories that have calibrated their measurements with the previous 40-3-2 soybean CRM (ERM-BF410n series) or another RM characterised by a 1:1 ratio between the DNA content of the GM and non-GM fraction are therefore expected to measure a much lower GM mass fraction in the new CRM than the certified value of 1.00 m/m %. The important message here is to not mix up the previous CRM batch with the new one, as both batches do not contain the same amount of transgenic copies. Despite this observation, the aim of using a CRM calibrant is to have a common reference point for the implementation of EU legislation on GMO thresholds and labelling[11].

4.4.3 Calculation of performance scores

Individual laboratory performance was expressed in terms of z and ζ scores in accordance with ISO 13528:2015[16], both calculated in the log domain as follows:

$$z = \frac{\log(x_i) - x_{pt-log}}{\sigma_{pt-log}} \quad (4)$$

$$\zeta = \frac{log(x_i) - x_{pt-log}}{\sqrt{u(x_{i-log})^2 + u(x_{pt-log})^2}} \quad (5)$$

where: x_i = the measurement result as reported by a participant;
$u(x_i)$ = the standard measurement uncertainty of the result reported;
$x_{pt\text{-}log}$ = the assigned value;
$u(x_{i\text{-}log})$ = the standard measurement uncertainty of the result reported;
$u(x_{pt\text{-}log})$ = the standard measurement uncertainty of the assigned value;
$\sigma_{pt\text{-}log}$ = the standard deviation for proficiency assessment.

For calculation of the ζ scores, the expanded uncertainties on the results reported by the laboratories were translated into standard measurement uncertainties [$u(x_i)$] using the coverage factor reported and converted to the log domain as follows (following general rules for the measurement uncertainty of $\log_{10}$-transformed values):

$$u(x_{i-log}) = 0.434 \ \frac{u(x_i)}{x_i} \quad (6)$$

When no measurement uncertainty was reported, it was set to zero ($u(x_i) = 0$). When no coverage factor was reported, k was set to 1.73 (assuming a rectangular distribution around the reported value with boundaries valuing ± U/√3 [17]).

Performance scores were calculated on the results as reported by the participants and rounded to one decimal afterwards. The interpretation of the z and ζ performance scores was done according to ISO 17043:2010[5]:

|score| ≤ 2.0	satisfactory performance;
2.0 < |score| < 3.0	questionable performance;
|score| ≥ 3.0	unsatisfactory performance.

The ***z* score** compares the participant's deviation from the assigned value with the standard deviation for proficiency assessment ($\sigma_{pt\text{-}log}$) used as common quality criterion. Measurements that are carried out correctly are assumed to generate results that can be described by a normal distribution with mean $x_{pt\text{-}log}$ and standard deviation $\sigma_{pt\text{-}log}$. The z scores will then be normally distributed with a mean of zero and a standard deviation of

1.0. Only 0.3 % of scores would be expected to fall outside the range -3.0 < z < 3.0 and only 5 % would be expected to fall outside the range -2.0 ≤ z ≤ 2.0. These percentages may change when the true interlaboratory variability deviates from the agreed standard deviation which was set to 0.10. It is unlikely that unacceptable z scores will occur by chance when no real problem exists; rather, it is likely that there is an identifiable cause for any anomaly when an unsatisfactory performance, expressed as a z score, is obtained.

The ***ζ* score** states whether the laboratory's result agrees with the assigned value within the respective measurement uncertainty. The denominator is the combined uncertainty of the assigned value [$u(x_{pt\text{-}log})$] and the measurement uncertainty as stated by the laboratory [$u(x_{i\text{-}log})$]. The ζ score includes all parts of a measurement result, namely the expected value (assigned value), its measurement uncertainty in the unit of the result as well as the uncertainty of the reported values. An unsatisfactory ζ score can either be caused by the presence of a significant bias (inaccurate measurement) or by a non-realistic estimation of the measurement uncertainty (seriously under-estimated), or by a combination of both. Participants that have obtained a satisfactory z score but an unsatisfactory ζ score may have underestimated their measurement uncertainty. Participants that have obtained an unsatisfactory z score but a satisfactory ζ score may have assessed the uncertainty of their result accurately but the result itself does not meet the performance expected for the PT scheme.

More detailed information about measurement uncertainty evaluation can be found in some international standards and other guidance documents[17,18,19,20,21].

4.4.4 Performance of the laboratories

The performance of the laboratories for GM quantification is primarily evaluated on the basis of their z scores. The ζ scores obtained are providing additional information to the laboratory regarding the correct estimation of the measurement uncertainty of the result, but should be used as indicative values only.

4.4.4.1 z scores

Table 9 summarises the performance results obtained in this PT, based on the z scores. Detailed results per laboratory are reported in Annex 4, Tables A4.1 and A4.2 and Figures A4.1 and A4.2.

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

Laboratory Performance	**Test Item 1**	**Test Item 2**
	40-3-2 Soybean	**40-3-2 Soybean**
Number of laboratories with $\|z\| \leq 2.0$ (satisfactory)	**67**	**78**
Number of laboratories with $2.0 < \|z\| < 3.0$ (questionable)	**9**	**3**
Number of laboratories with $\|z\| \geq 3.0$ (unsatisfactory)	**4**[a]	**2**

[a] One additional NRL/120 (L88) reported a value <LOQ, which is considered unacceptable.

A total of 4 laboratories obtained an unsatisfactory performance, expressed as z score, for quantification of event 40-3-2 in T1 (1 NRL/882 and 3 non-NRLs) and 2 laboratories for T2 (2 non-NRL). Another 5 NRL/882, 1 NRL/120 and 3 non-NRLs obtained a questionable z score for T1, and one NRL/882, one NRL/120 and one non-NRL similarly for T2. In case of an unsatisfactory performance obtained by an NRL the laboratory will be requested to perform a root-cause analysis and to communicate the outcome to the EURL GMFF, who will then follow-up with the laboratory.

One laboratory (L88) had reported that the 40-3-2 soybean mass fraction in T1 was below its LOQ of 0.04 m/m %. While "less than X" values were not included in the data evaluation, they were compared to the corresponding $x_{pt} - U(x_{pt})$ (after conversion to the log scale). Since the reported "less than X" value was lower than the corresponding $x_{pt} - U(x_{pt})$, the laboratory should have been able to quantify the analyte. Therefore, the laboratory statement was considered as unsatisfactory.

Although the performance scores were calculated on the log-transformed data, the corresponding GM mass fractions on the raw domain, which are easier to understand in daily laboratory practice, approximated the following values:

For 40-3-2 soybean in T1:

Assigned value on the raw domain	0.80 m/m %
$\|z\| \leq 2.0$ lower and upper limits	0.50 – 1.28 m/m %
$\|z\| < 3.0$ lower and upper limits	0.41 – 1.57 m/m %

For 40-3-2 soybean in T2:

Assigned value on the raw domain	0.76 m/m %
$\|z\| \leq 2.0$ lower and upper limits	0.48 – 1.22 m/m %
$\|z\| < 3.0$ lower and upper limits	0.39 – 1.50 m/m %

The general performance of the laboratories for quantification of event 40-3-2 soybean in both test items was very good. This event is one of the older GM events, inserted in the EU Register of authorised events in 1996, and is commonly found in feed samples on the global market. It is therefore not surprising that most laboratories are able to detect this event and to quantify it accurately. In a previous PT in 2014 (CT 02/14), which also included a chicken feed sample containing 40-3-2 soybean, the general performance of the participants was much worse (16 unsatisfactory *z* scores among 70 results, based on an agreed $\sigma_{pt\text{-}log}$ of 0.20). The latter outcome was due to issues with the extraction of good quality DNA from the highly processed matrix which contained the 40-3-2 soybean already before processing (*i.e.* the GM soybean was not spiked in). The chicken feed used in the current PT was from a different origin compared to the one used in CT 02/14, although also in this case the extracted DNA was, at least partially, degraded. In contrast, the DNA from the spiked 40-3-2 soybean was of high-molecular weight (see Section 2.1) and, therefore, easier to amplify during PCR. More important is that a considerable fraction of unprocessed non-GM soybean was added to the T1 mix to increase the total soybean content; the measurements on the DNA extracted from the latter material, presumably of good amplification quality, probably contributed mainly to the denominator (*i.e.* the taxon-specific DNA fraction) in the equation to express the GM content. The improved performance of the laboratories participating to the current PT may also be the result of the increased experience in the extraction of PCR-grade DNA from demanding sample matrices.

Most of the results reported for T2, which was a seed-based matrix, and therefore it was easier to extract good quality DNA, were close to the assigned value with its expanded measurement uncertainty; this can be seen in Figure A4.2 in Annex 4.

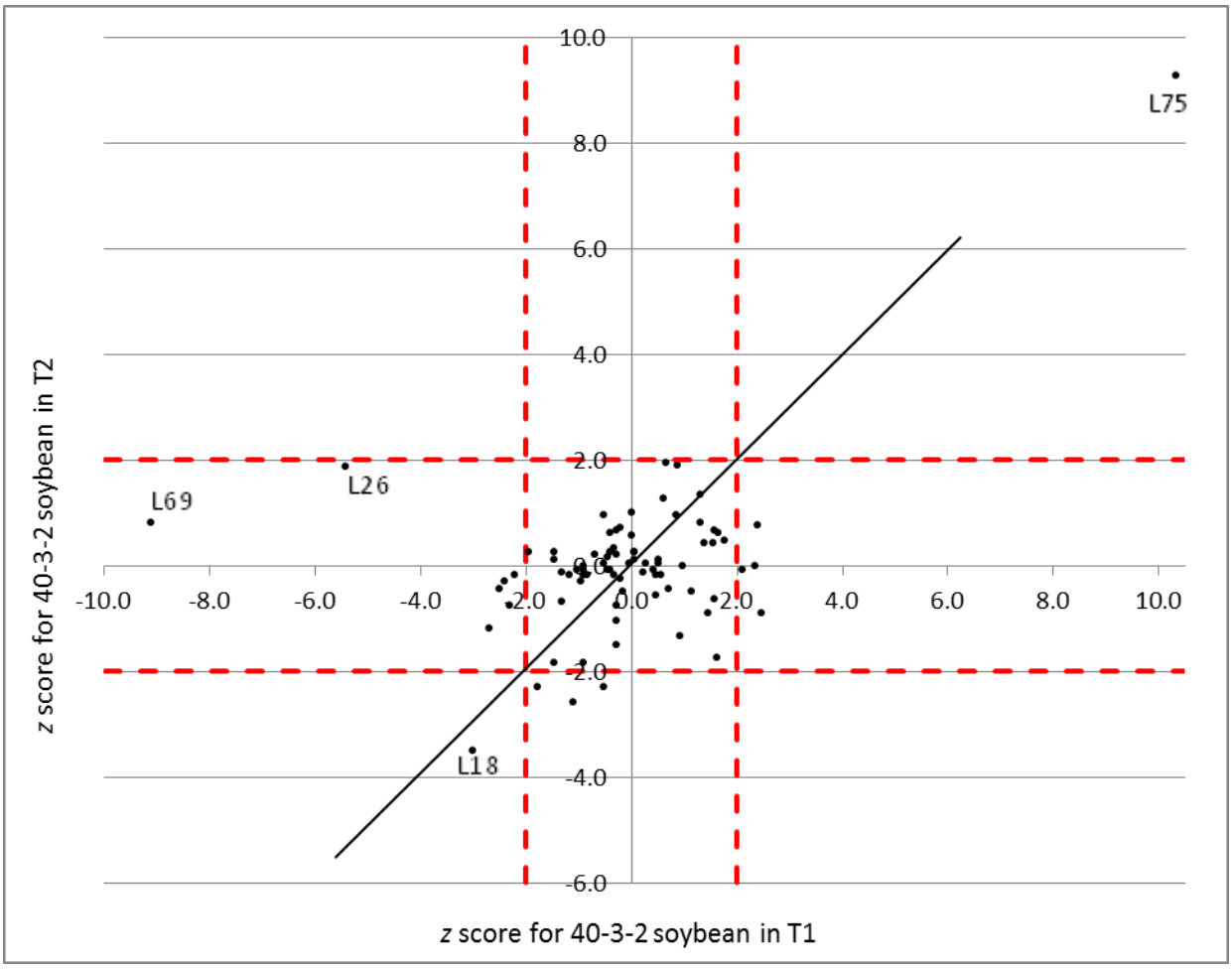
The participants to this PT were requested to quantify the same GM event in a compound feed (T1) on the one hand, and in a pure soybean material (T2) on the other hand. Figure 1 compares the performance of the laboratories to provide acceptable results for both tasks. The horizontal and vertical axes correspond to a *z* score of 0 for T1 and T2, respectively, while the dashed red lines indicate the limits of the satisfactory *z* scores ($|z|=2$). Points in the lower left or upper right quadrant, corresponding to L18 and L75, indicate participants who have a systematic bias in the application of the method. The points at the far-left of the x-axis (L26 and L69) represent participants that have had a problem particularly with T1, but not so much with T2; this may be related to the DNA extraction part of the workflow which was more challenging for T1 compared to T2. There are also other participants that may have had similar issues with T1, as more points lie outside the satisfaction interval on the x-axis compared to the y-axis.

For many but not all participants, there seem to be a slight tendency for consistent *z* scores for T1 and T2 (points along the diagonal line), which gives evidence of participant bias that affected both test items in a similar way. There are also a number of participants that have obtained a *z* score close to zero for both test items and which are represented by points close to where the horizontal and vertical axes cross, e.g. L05, L06, L09, L44,

L46, L50, L54, L55, L63, L64, L85, L87 and L89 (within $|z|<0.5$ for T1 and T2). These laboratories, 12 NRLs and one non-NRL, seem to have the whole analytical method, including DNA extraction and real-time PCR, very well under control for different types of samples. The highly proficient non-NRL used digital PCR for both measurements, indicating that this method also seems reliable for GM soybean quantification in different matrices.

Figure 1. Youden plot, comparing the *z* scores obtained by the participants for the determination of the 40-3-2 soybean mass fraction in T1 and T2.

The dashed red lines indicate the limits of satisfactory performance ($|z|\leq 2.0$) for each test item. The diagonal line displays the consistency of *z* scores in T1 and T2.



4.4.4.2 ζ scores

Tables A4.1 and A4.2 also report the ζ scores obtained by the laboratories as an informative evaluation for the laboratories of their result in combination with the reported uncertainty. A total of 54 laboratories were given a satisfactory performance, when expressed as ζ score, for quantification of 40-3-2 soybean in T1, 3 a questionable and 24 an unsatisfactory performance score (N = 81). For 40-3-2 soybean in T2 (N = 83), 62 laboratories performed satisfactorily, 5 questionable and 16 unsatisfactorily. As explained in Section 4.4.3, a bad ζ score may be due to a result that strongly deviates from the assigned value (and has therefore also yielded an unsatisfactory performance when expressed as a z score) or it may indicate an underestimation of the measurement uncertainty of the result.

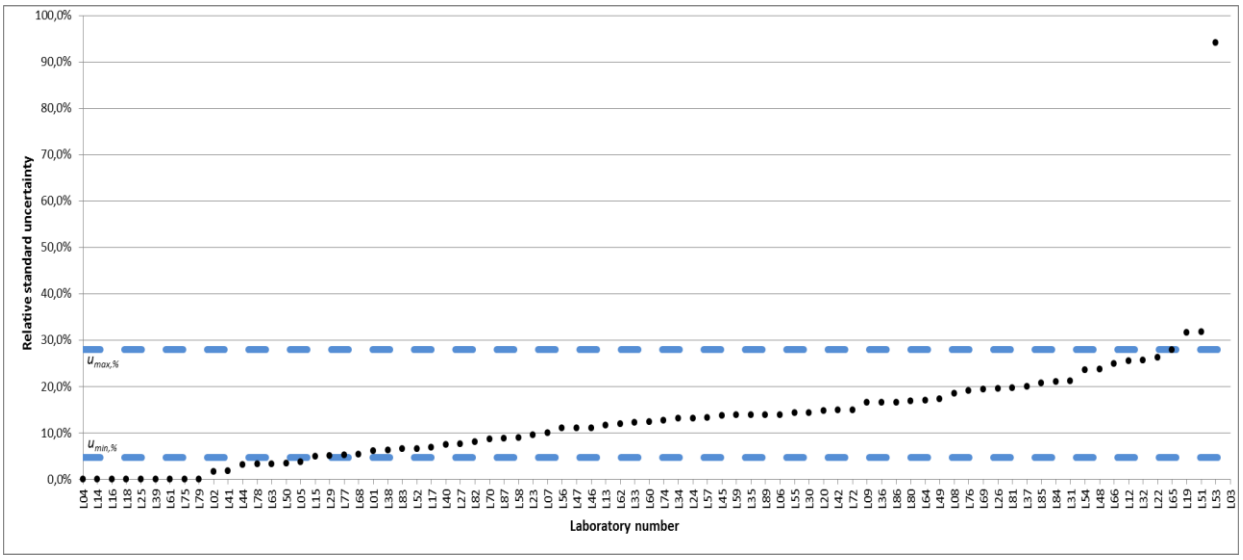
Figures A4.1 and A4.2 (Annex 4) allow verification if the reported measurement uncertainty bars overlap with the horizontal dashed (black) lines that delineate the satisfactory interval for the z scores. Laboratory L03 provided an expanded measurement uncertainty of 15.20 for T1 and T2, which is either strongly overestimated or corresponds to a relative uncertainty instead of the requested absolute uncertainty in m/m %. Also L53 has strongly overestimated its measurement uncertainty for the mass fraction of 40-3-2 soybean in T1 and T2, and L76 for T2 only, although they both obtained a satisfactory ζ score because their reported values were sufficiently close to the assigned value. From the same figures in Annex 4, it can easily be seen that several laboratories may have reported a rather low measurement uncertainty (or no uncertainty at all) and therefore received an unsatisfactory ζ score; e.g. the NRLs L01, L02, L40 and L78 would have obtained a satisfactory ζ score for 40-3-2 in T1 if they had reported a realistic measurement uncertainty (Figure A4.1). Similarly, for 40-3-2 in T2 (Figure A4.2), the results of the NRLs L38, L40, L41, L77, L78 and L83 would have been satisfactory if they had reported a realistic measurement uncertainty. Furthermore, nearly all laboratories that had not reported a measurement uncertainty value, and thus received an assumed uncertainty of zero, received an unsatisfactory ζ score. From the questionnaire it was noted that 32 laboratories (i.e. more than 1/3 of all participants) estimated the measurement uncertainty on the basis of the precision of the analysis replicates only; such an approach may not be sufficient to account for all analytical variability.

ISO 13528:2015[16] suggests to check whether a reported standard uncertainty (with a coverage factor k=1) is "realistic" and lies between a minimum and maximum uncertainty (u_{min} *and* u_{max}). This allows participants to review their reported uncertainty and evaluate if the reported uncertainty is counting all relevant components, or is over-counting some components. It is unlikely that a participant result will have a smaller standard uncertainty than the measurement uncertainty of the assigned value, so $u(x_{pt})$ can be used as a lower limit, called u_{min}. It is also unlikely that a participant reported standard uncertainty is larger than the robust standard deviation of the (NRL) results (u_{max}).

As an example, Figure 2 compares the relative standard uncertainties ($u_{i,\%}$) reported by the participants of this PT for T1, calculated from the expanded uncertainty U and reported k factor, and expressed as percentage of the reported result. On the raw data scale, $u_{min,\%}$ and $u_{max,\%}$ for the 40-3-2 soybean mass fraction in T1 correspond to 5 % and 28 %, respectively. Therefore, a standard measurement uncertainty smaller than 0.04 m/m % is probably underestimated, while a standard uncertainty above 0.23 m/m % may be overestimated. However, these are informative indicators only. Measurement uncertainties below u_{min} or above u_{max} can be valid, and in such case the laboratory should check the result or the uncertainty estimate.

Figure 2. Reported relative standard uncertainties ($u_{i,\%}$) for T1.

The horizontal blue lines refer to $u_{min,\%}$ and $u_{max,\%}$ as defined in the text. Note that the relative uncertainty of L03 is out of scale and not shown. Laboratories that failed to report a measurement uncertainty were given a zero value.



5 Conclusions

Participants in this PT were required to analyse two test items varying in composition and complexity, but containing the same GM event. The analytical tasks resembled the routine operational analysis tasks of an official control laboratory analysing a food or feed material for the presence of material derived from, containing, or consisting 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.

A large majority of the participants performed satisfactorily for the tasks in this PT, *i.e.* the detection and quantification of the soybean event 40-3-2 in T1, a chicken feed powder, and in T2, a soybean flour. All participants who tested for the events were able to identify the correct event in both test items. Regarding quantification, four laboratories, including one NRL/882 and 3 non-NRLs, obtained an unsatisfactory *z* performance score for the 40-3-2 soybean measurements in the more difficult feed matrix. One NRL/120 reported an unacceptable "<LOQ" result for the 40-3-2 soybean mass fraction in T1, but the reported result for T2 was satisfactory. Two of the non-NRL laboratories were also unsatisfactory for the quantification of the same event in the T2 matrix.

It is recommended for several laboratories to re-consider the estimation of their measurement uncertainty in order to report a more realistic uncertainty and, consequently, to obtain a satisfactory ζ performance score.

Acknowledgements

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Body [1]	Organisation	Department	City	Country
NRL/882	AGES-Institute for Food Safety Vienna		Vienna	AUSTRIA
	Umweltbundesamt GmbH		Vienna	AUSTRIA
	CRA-W - Walloon Agricultural Research Center	Valorization of agric. prod.	Gembloux	BELGIUM
	Institute for Agricultural and Fisheries Research	Technology and Food - PI	Merelbeke	BELGIUM
	Scientific Institute of Public Health (WIV-ISP)	PBB - GMOlab	Brussels	BELGIUM
	National Center of Public Health and Analyses	GMO	Sofia	BULGARIA
	Croatian Institute of Public Health		Zagreb	CROATIA
	State General Laboratory	GMOs and Allergens	Nicosia	CYPRUS
	Crop Research Institute		Prague	CZECH REPUBLIC
	Danish Veterinary and Food Administration	Food Chem. and Plant Health	Ringsted	DENMARK
	Finnish Customs Laboratory		Espoo	FINLAND
	BioGEVES		Surgeres	FRANCE
	Service Commun des Laboratoires		Illkirch Graffenstad	FRANCE
	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	Referat 503	Berlin	GERMANY
	General Chemical State Laboratory	A' Chemical Service of Athens	Athens	GREECE
	National Food Chain Safety Office		Budapest	HUNGARY
	Istituto Zooprofilattico Lazio e Toscana	Biotechnology Unit	Rome	ITALY
	Institute of Food Safety, Animal Health and Environment „BIOR"		Riga	LATVIA
	National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO	Vilnius	LITHUANIA
	Laboratoire National de Santé	food control	Dudelange	LUXEMBOURG
	RIKILT Wageningen University & Research		Wageningen	NETHERLANDS
	Instytut Zootechniki PIB	KLP Szczecin	Szczecin	POLAND
	National Veterinary Research Institute		Pulawy	POLAND
	Regional Laboratory of Genetically Modified Food		Tarnobrzeg	POLAND
	Instituto Nacional de Investigação Agrária e Veterinária	UEIS-SAFSV	Oeiras	PORTUGAL
	Institute for Diagnosis and Animal Health	Molecular Biology and GMOs	Bucharest	ROMANIA
	State Veterinary and Food Institute, VFI in Dolny Kubin		Dolny Kubin	SLOVAKIA
	Central Control and Testing Institute of Agriculture, Bratislava	OMB NRL	Bratislava	SLOVAKIA
	National Institute of Biology		Ljubljana	SLOVENIA
	Laboratorio Arbitral Agroalimentario LAA-MAPAMA	OGM	Madrid	SPAIN
	Centro Nacional De Alimentaciòn (Agencia Española De Consumo, Seguridad Alimentaria Y Nutriciòn)	Biotechnology Unit	Madrid	SPAIN
	National Food Agency		Uppsala	SWEDEN
	LGC		Teddington	UNITED KINGDOM
NRL/120	Finnish Food Safety Authority Evira		Helsinki	FINLAND
	Thüringer Landesamt für Verbraucherschutz (TLV)	Lebensmittelsicherheit	Bad Langensalza	GERMANY
	LAVES-Lebensmittel- und Veterinärinstitut Braunschweig/Hannover		Braunschweig	GERMANY
	Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Amtliche Lebensmitteluntersuchung	Dresden	GERMANY
	BfR	Food Safety	Berlin	GERMANY
	Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	Halle	GERMANY
	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V (LALLF MV)	200/PCR	Rostock	GERMANY
	Institut für Hygiene und Umwelt Hamburg	Gentechniküberwachungslabor	Hamburg	GERMANY
	LUFA Speyer	Referat II/2	Speyer	GERMANY
	CVUA Freiburg	GMO	Freiburg	GERMANY
	Bavarian Health and Food Safety Authority (LGL)		Oberschleissheim	GERMANY
	LTZ Augustenberg		Karlsruhe	GERMANY
	LLBB		Berlin	GERMANY
	Landeslabor Schleswig-Holstein		Neumünster	GERMANY
	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GB 6, Fachbereich 63	Nossen	GERMANY

Body	Organisation	Department	City	Country
NRL/120 cont.	Istituto Superiore di Sanità	DSPVSA	Rome	ITALY
	CREA-SCS	Sede di Tavazzano, Laboratorio	Tavazzano (LO)	ITALY
	Netherlands Food and Consumer Product Safety Authority (NVWA)	Laboratorium VV	Wageningen	NETHERLANDS
	Plant Breeding and Acclimatization Institute NRI	GMO Controlling Laboratory	Blonie	POLAND
	Fera Science Ltd	Plants	York	UNITED KINGDOM
	SASA Scottish Government	Seed certification	Edinburgh	UNITED KINGDOM
Non-NRL	FASFC Melle	GMO	Melle	BELGIUM
	Laboratório Nacional Agropecuário - LANAGRO/MG		Pedro Leopoldo/MG	BRAZIL
	Ministry of Agriculture, Livestock and Food Supply	Official Laboratory of Goiás	Goiania	BRAZIL
	Laboratory of SGS Bulgaria Ltd		Varna	BULGARIA
	Servicio Agrícola y Ganadero	Biotechnology	Santiago	CHILE
	Instituto Nacional de Vigilancia de Medicamentos y Alimentos Invima	Laboratorio OGM	Bogotá	COLOMBIA
	Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Seed and Seedlings	Non-NRL	Osijek	CROATIA
	CVUA-OWL		Detmold	GERMANY
	Thüringer Landesanstalt für Landwirtschaft		Jena	GERMANY
	Biomi Ltd.		Godollo	HUNGARY
	ICAR-National Bureau of Plant Genetic Resources	Division of Genomic Resources	New Delhi	INDIA
	IZSLER		Brescia	ITALY
	Istituto Sperimentale Del Piemonte, Liguria e Valle D'Aosta	S.C. Biotechnologie	Torino	ITALY
	Istituto Zooprofilattico Sperimentale Abruzzo e Molise	Hygiene in Food Technology	Teramo	ITALY
	American University of Science and Technology	Laboratory Science & Technology	Ashrafieh-Beirut	LEBANON
	SENASICA-CNRDOGM	Detección de OGM	Tecámac	MEXICO
	Bureau of Plant Industry, National Plant Quarantine Services Division, Post Entry Quarantine Station	Department of Agriculture	Los Banos, Laguna	PHILIPPINES
	Laboratorul Central pentru Calitatea Semintelor si a Materialului Saditor Bucuresti	LEDOMG	Bucuresti	ROMANIA
	Institute of Molecular Genetics and Genetic Engineering	Plant Molecular Biology	Belgrade	SERBIA
	SP Laboratorija a.d.	Genetical dpt.	Becej	SERBIA
	A Bio Tech Lab	Laboratory for biotechnology	Sremska Kamenica	SERBIA
	Agri-Food & Veterinary Authority of Singapore	Veterinary Public Health Labor	Singapore	SINGAPORE
	Federal Food Safety and Veterinary Office FSVO	Risk Assessment Division	Bern	SWITZERLAND
	Agroscope	Feed Analytics	Posieux	SWITZERLAND
	Ankara Food Control Laboratory	Molecular Biology	Ankara	TURKEY
	State Scientific Research Institute of laboratory Diagnostic and Veterynary Sanitary Expertise	Research GMOs Department	Kyiv	UKRAINE
	Ukrmetrteststandart	Molecular Biology	Kiev	UKRAINE
	Ukrainian Laboratory of Quality and Safety of Agricultural Products (ULQSAP)		Chabany village	UKRAINE
	USDA-GIPSA	Biotechnology Laboratory	Kansas City	UNITED STATES
	Agricultural Genetics Institute	GMO Detection	04	VIETNAM
	National Institute for Food Control	Quality management	Ha Noi	VIETNAM
	Quality Assurance and Testing Center 3 (QUATEST 3)	Microbiology – GMO Testing Lab	Bienhoa	VIETNAM

[1] **NRL/882** means NRLs designated by their Member State to coordinate the activities of official laboratories for GMO control under Regulation (EC) No 882/2004;
NRL/120 means NRLs nominated under Regulation (EU) No 120/2014 to support the EURL GMFF on method validation (and not also NRL/882);
Non-NRL means official control laboratories from EU or non-EU countries that are not NRLs according to the Regulations mentioned above.

References

(including those referred to in the Annexes)

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

2. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Union* L 191: 1-52

3. Commission Implementing Regulation (EU) No 120/2014 of 7 February 2014 amending Regulation (EC) No 1981/2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and the Council as regards the Community reference laboratory for genetically modified organisms. *Off. J. Eur. Union* L 39: 46-52

4. Commission Regulation (EU) No 619/2011 of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorisation procedure is pending or the authorisation of which has expired. *Off. J. Eur. Union* L 166: 9-15

5. ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing. International Organization for Standardization, Geneva, Switzerland

6. Dimitrievska, B., Kortekaas, A.M., Seghers, J., Leys, H., Corbisier, Ph., Trapmann, S. (2017). The certification of different mass fractions of MON-Ø4Ø32-6 in soya bean powder. Certified Reference Materials ERM®-BF410ap, ERM®-BF410bp, ERM®-BF410cp, ERM®-BF410dp and ERM®-BF410ep. EC certification report EUR 28665 EN, ISBN 978-92-79-70107-8

7. Rosa, S.F., Gatto, F., Angers-Loustau, A., Petrillo, M., Kreysa, J., Querci, M. (2016). Development and applicability of a ready-to-use PCR system for GMO screening. *Food Chemistry* 201: 110–119

8. Querci, M., Foti, N., Bogni, B., Kluga, L., Broll, H. & Van den Eede, G. (2009). Real-time PCR-based ready-to-use multi-target analytical system for GMO detection. *Food Anal. Methods* 2: 325-336

9. ISO 17034:2016 General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland

10. Technical guidance document from the European Union Reference Laboratory for Genetically Modified Food and Feed on the implementation of Commission Regulation (EU) No 619/2011 (http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm)

11. Corbisier P., Barbante A., Berben G., Broothaerts W., De Loose M., Emons H., Georgieva Tz, Lievens A., Mazzara M., Papazova N., Perri E., Sowa S., Stebih D., Terzi V., Trapmann S. (2017). Recommendation for the unit of measurement and the measuring system to report traceable and comparable results expressing GM content in accordance with EU legislation. JRC Technical report (http://gmo-crl.jrc.ec.europa.eu/ENGL/docs/WG-UoM-Final-Report.pdf)

12. Analytical Methods Committee (1989). Robust statistics – How not to reject outliers Part 1. Basic Concepts. *Analyst* 114: 1359-1364

13. Analytical Methods Committee (2001). Robust statistics: a method for coping with outliers. *AMC Technical Brief.* No. 6. April 2001

14. Thompson, M., Ellison, S.L.R., Owen, L., Mathieson, K., Powell, J., Key, P., Wood, R., Damant, A.P. (2006). Scoring in Genetically Modified Organism Proficiency Tests Based on Log-Transformed Results. *J. AOAC Int.* 89: 232-239

15. Analytical Methods Committee (2004). GMO Proficiency Testing: Interpreting *z* scores derived from log-transformed data. RSC. *AMC Technical Brief.* No. 18. December 2004.

16. ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison. International Organization for Standardization, Geneva, Switzerland

17. Ellison, S.L.R., Williams, A. (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition (2012), ISBN 978-0-948926-30-3. Available from www.eurachem.org.

18. ISO/IEC Guide 98-3:2008 (JCGM/WG1/100) Uncertainty of measurement - Part 3: Guide to the expression of uncertainty in measurement

19. Linsinger, T. (2010). Comparison of a measurement result with the certified value. Application Note 1. Luxemburg, Office for Official Publications of the European Communities (https://crm.jrc.ec.europa.eu)

20. Trapmann, S., Burns, M., Broll, H., Macarthur, R., Wood, R., Zel, J. (2009). Guidance Document on Measurement Uncertainty for GMO Testing Laboratories. Luxembourg, Office for Official Publications of the European Communities, EUR 22756 EN/2-2009

21. Corbisier, P, Zobell, O., Trapmann, S., Auclair, G. and Emons, H. (2014). Training Manual on GMO Quantification: Proper Calibration and Estimation of Measurement Uncertainty. Luxembourg, Publications Office of the European Union (doi:10.2787/10085)

22. Thompson, M., Wood, R. (1993). The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories. *J. AOAC Int.* 76: 926-940

23. Powell, J., Owen, L. (2002). Reliability of Food Measurements: The Application of Proficiency Testing to GMO Analysis. *Accred. Qual. Ass.* 7: 392-402

24. Linsinger, T.P.J., van der Veen, A.M.H., Gawlik, B.M., Pauwels, J., Lamberty, A. (2004). Planning and combining of isochronous stability studies of CRMs. *Accred. Qual. Assur.* 9: 464-472

Annexes

Annex 1. Homogeneity and stability of test items

A1.1 Homogeneity of test items

The homogeneity of T2 was confirmed during the certification of this CRM.

The assessment of the homogeneity[16] of T1 was performed after the test item had been packed in its final form and before distribution to participants, using the following acceptance criterion:

$$s_s \leq 0.3\sigma_{pt} \qquad \text{(A1.1)}$$

Where s_s is the between-test item standard deviation as determined by a 1-way random effects ANOVA[22] and σ_{pt} is the standard deviation for comparative testing. The value of σ_{pt}, the target standard deviation for proficiency assessment, was based upon the experience acquired in previous PTs, and set to 0.10 on the log domain[23].

If the criterion according to A1.1 is met (i.e. $s_s \leq 0.030$), the between-test item standard deviation contributes no more than about 10 % to the standard deviation for comparative testing.

The repeatability of the test method is the square root of the mean sum of squares within-test items MS_{within}. The relative between-test item standard deviation $s_{s,rel}$ is given by

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

where: $MS_{between}$ is the mean sum of squares between test items
MS_{within} is the mean sum of squares within test items
n is the number of replicates for each sample
$\bar{y}$ is the mean of the homogeneity data

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

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

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

Seven bottles (N = 7) were randomly selected and analysed in five replicates (n = 5). The between-test item standard deviation was 0.018 m/m %. The criterion described in formula (A1.1) was fulfilled (0.018 < 0.030), indicating that T1 was adequately homogeneous.

A1.2 Stability of test items

For T1, an isochronous short-term stability study[24] 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. The 40-3-2 soybean mass fraction was measured by qPCR. The measurements were performed under intermediate precision conditions with respect to the PCR plates.

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 the soybean event 40-3-2 mass fraction. Even at 60 °C, no significant trend was measured, although the extracted DNA was more fragmented as seen by agarose gel electrophoresis.

The test items were shipped at ambient temperature.

The stability of T1 during the period covered by the PT (approximately 5 months between production of the test item and the deadline for results reporting) was tested by analysing, simultaneously on one PCR plate, two units (N = 2, n = 3) stored either at the normal storage temperature (4 °C) or at a reference temperature (-70 °C). The evaluation was based on the results ratio between samples stored at 4 °C and -70 °C. The data were evaluated against storage time and regression lines were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage). No significant trend was detected at a 95 % confidence level. The T1 material can, therefore, be stored at 4 °C and was stable during the period covered by this CT.

The stability of T2 was ensured as part of the post-certification stability monitoring of ERM-BF410p. Measurements were performed simultaneously on one PCR plate as described for T1, on units stored at the normal storage temperature (4 °C) and at a reference temperature (-70 °C). No significant trend was detected at a 95 % confidence level. The T2 material can, therefore, be stored at 4 °C and was stable during the period covered by this CT.

Annex 2: Questionnaire data

The results received from 82 laboratories were exported from the EUSurvey "Questionnaire on CT 02/17 analysis" and are tabulated below. Multiple answers were allowed for all questions, except for the questions on the calibrant used. The results of the open questions were manually analysed and reported. Answers to the questions on GM events that were not to be quantified in the test items are not shown.

Select the group to which your organisation belongs. Note: 882 and 120 refer to EU Regulations 882/2004 and 120/2014, resp.; select NRL/120 if your organisation is ONLY listed under Regulation 120/2014; select non-NRL if your organisation is not an NRL under either EU Regulation.

		Answers	Ratio
NRL/882		33	40.2%
NRL/120		20	24.4%
Non-NRL		29	35.4%
No Answer		0	0%

T1: Please select the option that applies and proceed with the questionnaire.

		Answers	Ratio
T1 was not analysed: go to Q1		2	2.44%
T1 was analysed: go to Q2		80	97.6%
No Answer		0	0%

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

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		1	1.22%
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.)		1	1.22%
g) Other reason		0	0%
No Answer		80	97.6%

T1: 2. Select the DNA extraction method used for T1

		Answers	Ratio
CTAB		41	50%
NucleoSpin Food		10	12.2%
NucleoSpin Plant		3	3.66%
GeneSpin		4	4.88%
Promega Wizard		3	3.66%
DNeasy Plant		3	3.66%
DNeasy Mericon Food		5	6.1%
Biotecon Foodproof		5	6.1%
SDS		4	4.88%
Fast ID Genomic DNA		2	2.44%
Maxwell 16 Plant DNA		0	0%
Maxwell 16 Food, Feed, Seed		5	6.1%
Generon Ion Force		1	1.22%
Other		2	2.44%
No Answer		2	2.44%

T1: 3. Select any additional DNA purification method used for T1.

		Answers	Ratio
No additional clean-up		51	62.2%
Additional ethanol precipitation		9	11.0%
Eurofins DNAExtractor cleaning column		3	3.66%
Promega Wizard DNA clean-up resin		6	7.32%
Qiagen QIAQuick		4	4.88%
Qiagen Genomic-Tip 20/G		1	1.22%
Other method (no need to specify)		7	8.54%
No Answer		2	2.44%

T1: 4. Indicate the number of replicate DNA extractions used to obtain the results.

		Answers	Ratio
1		0	0%
2		52	63.4%
3		9	11.0%
4		10	12.2%
5		2	2.44%
6		6	7.32%
>6		1	1.22%
No Answer		2	2.44%

T1: 5. Select the approach(es) used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		6	7.32%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		40	48.8%
We verify that the amplification curves look normal		24	29.3%
We run two dilutions and verify if the delta Cq or GM% are as expected		31	37.8%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		13	15.8%
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	19.5%
We add an internal positive control to the reactions and check the Cq		15	18.3%
Other		1	1.22%
No Answer		2	2.44%

T1: 6. Select the option applicable to your analysis for 40-3-2 soybean in T1?

		Answers	Ratio
Not tested		0	0%
Detected but not quantified		3	3.66%
Detected and quantified: please fill in Q6a-6h		77	93.9%
Found absent		0	0%
No Answer		2	2.44%

T1: 7. Select the option applicable to your analysis for 68416 soybean in T1?

		Answers	Ratio
Not tested		14	17.1%
Detected but not quantified		2	2.44%
Detected and quantified: please fill in Q7a-7h		0	0%
Found absent		64	78.1%
No Answer		2	2.44%

T1: 8. Select the option applicable to your analysis for MON89788 soybean in T1?

		Answers	Ratio
Not tested		6	7.32%
Detected but not quantified		33	40.2%
Detected and quantified: please fill in Q8a-8h		15	18.3%
Found absent		26	31.7%
No Answer		2	2.44%

T1: 9. 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		45	54.9%
b) The event-specific detection method is not validated in our laboratory		7	8.54%
c) Reference material, primers, probes, or other reagents were not available (in time)		9	11.0%
d) The result obtained was below the LOD/LOQ		18	22.0%
e) Practical constraints (instrument broken, no personnel, etc.)		0	0%
f) Other reason		9	11.0%
No Answer		2	2.44%

T1: 6.a. Soybean 40-3-2: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		68	82.9%
Delta Cq method (one calibration curve)		9	11.0%
Digital PCR (no calibration curve)		2	2.44%

No Answer		5	6.1%

T1: 6.b. Select the calibrant used for the 40-3-2 standard curve.

		Answers	Ratio
CRM from JRC (ex-IRMM), certified in GM mass fraction (g/kg)		71	86.6%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction (g/kg or m/m %)		1	1.22%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		4	4.88%
No calibrant used, digital PCR done		1	1.22%
No Answer		5	6.1%

T1: 6.c. Select the endogenous target(s) used for relative quantification of 40-3-2 soybean in T1.

		Answers	Ratio
Soybean lec 74 bp (40-3-2, MON89788, MON87701, 44406, 356043, 305423, etc.)		65	79.3%
Soybean lec 81 bp (Pauli et al., 2001)		5	6.1%
Soybean lec 102 bp (A5547, FG72)		1	1.22%
Soybean lec 105 bp (A2704)		0	0%
Soybean lec 118 bp (Shindo et al., 2002)		5	6.1%
Other, please specify below		1	1.22%
No Answer		5	6.1%

Specify the reference target(s) used (if different from above):
SOJA LEKTIN 80 bp - Va M, Pijnenburrg H, Gendre F, Brignon P (1999) J Agric Food Chem 47:5261-5266

T1: 6.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 40-3-2. 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		59	72.0%
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)		13	15.9%
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.		0	0%
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.		2	2.44%
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 %.		3	3.66%
No Answer		5	6.1%

Conversion factor used to turn results into m/m %, if applicable, and/or clarification on preparation of standards.
1
Conversion factor of 1

T1: 6.e. What was the amount of sample DNA (ng) used per PCR for 40-3-2. 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		12	14.6%
250 ng		5	6.1%
200 ng		30	36.6%
150 ng		9	11.0%
100 ng		18	22.0%
50 ng		14	17.1%
25 ng		6	7.32%
15 ng		0	0%
<10 ng		0	0%
No Answer		5	6.1%

T1: 6.f. What was the LOQ (in m/m %) for the 40-3-2 quantification?

	Answers
0.01	3
0.02	4
0.03	1
0.04	6
0.05	7
0.06	2
0.08	4
0.09	7
0.1	37
0.19	1
0.2	1
0.3	1
0.35	1
0.4	1
1.3	1

T1: 6.g. How was the LOQ for 40-3-2 determined (if applicable)?

		Answers	Ratio
Determined from the qPCR analysis for the current sample		20	24.4%
Determined during the in-house validation of the method		42	51.2%
Taken from the EURL GMFF validation report		17	20.7%
By another approach, please explain below		2	2.44%
No Answer		5	6.1%

Explanation on alternative LOQ determination:
Determined from the digital PCR analysis for the current sample
Information about LOQ introduced by the manufacturer (R-Biopharm) of diagnostic kit
The u was obtain through the estimation of the sd taking into account the repeatability and intermediate precision associated with the test sample. Coverage factor k = 2 was applied then.

T1: 6.h. How did you estimate the measurement uncertainty on the result reported for 40-3-2 soybean?

		Answers	Ratio
Uncertainty budget (ISO GUM)		2	2.44%
Uncertainty of the method (in-house validation)		27	32.9%
Known uncertainty of the standard method		9	11.0%
Measurement of replicates (precision)		32	39.0%
From interlaboratory comparison data		4	4.88%
Estimation based on judgement		2	2.44%
In another way, please specify below		6	7.32%
No Answer		5	6.1%

Explanation on alternative determination of measurement uncertainty:
U=S/a √1/p+1/n+(c0-c)2/Sxx
Uncertainty=Coverage Factor (P=95% anf f=n-1) * Standard Deviation / Square-root (Number of Measurements)
MU was estimated according to the Guidance Document on MU for GMO Testing Laboratories JRC ISSN 1018-5593
we used a calculated k-factor based on the number of repeats
Estimation based on within laboratory reproducibility
95% confidence interval of the results for the current sample
The u was obtain through the estimation of the sd taking into account the repeatability and intermediate precision associated with the test sample. Coverage factor k = 2 was applied then.
Combined Uncertainty (CRM + measurement) following Application note (Lingsinger, 2005, JRC Geel)

Additional comments and suggestions
MON89788 soybean was detected but not quantified because results for this event were < LOQ
We saw in the qPCR for MON89788 Ct-values at 37 - 39, but < LOD (< 0.02 %).
Additional tests with one extract performed with Nucleospin Food Kit and with one extract performed with CTAB+Nucleospin Food Kit showed no significant differences in event detection and GTS 40-3-2 quantification (examined with digital PCR)
T1:9 not all detected GM were asked to be quantified
For T1 trace amounts of MON89788 were detected either in qualitative screening as well as in quantification. The given/calculated GM % (m/m) of 0.02 % is well below the known and validated LOQ of the method which is 0.1 %.
We detected a low level of MON89788 but as this quantified at 0.006%, lower than the threshold for reporting, we took this as a negative result.
According course material of JRC: GMO Quantification: Proper calibration and Estimation of Measurement Uncertainty
MU is based on intermediate precision data

The sample was strongly inhibited MON89788 was detected under LOQ
In T1 the measured GM% for MON89788 of 0.01% is below the determined LOQ (0.2%) therefore no MU was reported.
MON89788 was detected in traces at the LOD
MON89788 SOY result obtained below the LOQ, but copy numbers of MON89788 was above LOD.
T1 was inhibited. A 10x dilution of the DNA was needed to get a reasonable result.
DNA extraction of T1 has conducted using DNA extraction kits SureFood Prep Basic (S1052) and SureFood Prep Advanced (S1053), R-Biopharm AG. Identification of Soya GM-lines was conducted using diagnostic kits SureFood GMO ID Roundup Ready Soya (S2030) and SureFood GMO ID RR2Y (S2034). Quantification kits were SureFood GMO Quant Roundup Ready Soya (S2014), SureFood GMO Quant 35S soya (S2028) and SureFood GMO Quant RR2Y Soya (S2029), R-Biopharm AG
MON89788 soybean was detected in sample T1 but only in some cases - 2 PCR replicates were always positive out of 4 after several repetitions.
Remark (precision) in relation to T1.8 and T1.9: MON89788 soybean was detected in the sample but not quantified because of < LOQ

T2: Please select the option that applies and proceed with the questionnaire.

		Answers	Ratio
T2 was not analysed: go to Q1		0	0%
T2 was analysed: go to Q2		82	100%
No Answer		0	0%

T2: 1. Why did you not analyse test item 2?

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		0	0%
b) The methods are not validated in our laboratory		0	0%
c) We could not obtain sufficient good quality DNA suitable for further analysis		0	0%
d) Reference material, primers, probes, or other reagents were not available (in time)		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		82	100%

T2: 2. Select the DNA extraction method used for T2.

		Answers	Ratio
CTAB		40	48.8%
NucleoSpin Food		11	13.4%
NucleoSpin Plant		3	3.66%
GeneSpin		4	4.88%
Promega Wizard		3	3.66%
DNeasy Plant		3	3.66%
DNeasy Mericon Food		4	4.88%
Biotecon Foodproof		5	6.1%
SDS		4	4.88%
Fast ID Genomic DNA		3	3.66%
Maxwell 16 Plant DNA		0	0%
Maxwell 16 Food, Feed, Seed		4	4.88%
Generon Ion Force		1	1.22%
Other		2	2.44%
No Answer		0	0%

T2: 3. Select any additional DNA purification method used for T2.

		Answers	Ratio
No additional clean-up		52	63.4%
Additional ethanol precipitation		9	11.0%
Eurofins DNAExtractor cleaning column		3	3.66%
Promega Wizard DNA clean-up resin		6	7.32%
Qiagen QIAQuick		5	6.1%
Qiagen Genomic-Tip 20/G		1	1.22%
Other method (no need to specify)		8	9.76%
No Answer		0	0%

T2: 4. Indicate the number of replicate DNA extractions used to obtain the results.

		Answers	Ratio
1		0	0%
2		56	68.3%
3		9	11.0%
4		10	12.2%
5		2	2.44%
6		4	4.88%
>6		1	1.22%
No Answer		0	0%

T2: 5. Select the approach(es) used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		7	8.54%
We run two dilutions and verify if the delta Cq or GM% are as expected		32	39.0%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		12	14.6%
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		15	18.3%
We add an internal positive control to the reactions and check the Cq		16	19.5%
We verify that the amplification curves look normal		25	30.5%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		36	43.9%
Other		1	1.22%
No Answer		0	0%

T2: 6. Select the option applicable to your analysis for 40-3-2 soybean in T2?

		Answers	Ratio
Not tested		0	0%
Detected but not quantified		2	2.44%
Detected and quantified: please fill in Q6a-6h		80	97.6%
Found absent		0	0%
No Answer		0	0%

T2: 7. Select the option applicable to your analysis for 68416 soybean in T2?

		Answers	Ratio
Not tested		15	18.3%
Detected but not quantified		1	1.22%
Detected and quantified: please fill in Q7a-7h		0	0%
Found absent		66	80.5%
No Answer		0	0%

T2: 8. Select the option applicable to your analysis for MON89788 soybean in T2?

		Answers	Ratio
Not tested		5	6.1%
Detected but not quantified		6	7.32%
Detected and quantified: please fill in Q8a-8h		3	3.66%
Found absent		68	82.9%
No Answer		0	0%

T2: 9. 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		61	74.4%
b) The event-specific detection method is not validated in our laboratory		6	7.32%
c) Reference material, primers, probes, or other reagents were not available (in time)		10	12.2%
d) The result obtained was below the LOD/LOQ		5	6.1%
e) Practical constraints (instrument broken, no personnel, etc.)		0	0%
f) Other reason		2	2.44%
No Answer		0	0%

T2: 6.a. 40-3-2: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		71	86.6%
Delta Cq method (one calibration curve)		9	11.0%
Digital PCR (no calibration curve)		2	2.44%

No Answer		2	2.44%

T2: 6.b. Select the calibrant used for the 40-3-2 standard curve.

		Answers	Ratio
CRM from JRC (ex-IRMM), certified in GM mass fraction (g/kg)		74	90.2%
CRM from IRMM, certified in GM copy number ratio (plasmid CRM)		0	0%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction (g/kg or m/m %)		1	1.22%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g.		4	4.88%
No calibrant used, digital PCR done		1	1.22%
No Answer		2	2.44%

T2: 6.c. Select the endogenous target(s) used for relative quantification of 40-3-2 soybean in T2.

		Answers	Ratio
Soybean lec 74 bp (40-3-2, MON89788, MON87701, 44406, 356043, 305423, etc.)		63	76.8%
Soybean lec 81 bp (Pauli et al., 2001)		5	6.1%
Soybean lec 102 bp (A5547, FG72)		1	1.22%
Soybean lec 105 bp (A2704)		0	0%
Soybean lec 118 bp (Shindo et al., 2002)		6	7.32%
Other, please specify below		2	2.44%
No Answer		5	6.1%

Specify the reference target(s) used (if different from above):
Lectin - 74bp
lec 74bp
Lectine
SOJA LEKTIN 80 bp Va M, Pijnenburrg H, Gendre F, Brignon P (1999) J Agric Food Chem 47:5261-5266
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.

T2: 6.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 40-3-2. 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		61	74.4%
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	17.1%
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.		0	0%
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.		2	2.44%
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 %.		3	3.66%
No Answer		2	2.44%

Conversion factor used to turn results into m/m %, if applicable, and/or clarification on preparation of standards.
1
Conversion factor of 1

T2: 6.e. What was the amount of sample DNA (ng) used per PCR for 40-3-2. 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		12	14.6%
250 ng		5	6.1%
200 ng		30	36.6%
150 ng		7	8.54%
100 ng		21	25.6%

50 ng		17	20.7%
25 ng		7	8.54%
15 ng		2	2.44%
<10 ng		0	0%
No Answer		2	2.44%

T2: 6.f. What was the LOQ (in m/m %) for the 40-3-2 quantification?

	Answers
0.01	5
0.02	6
0.03	1
0.04	7
0.05	7
0.06	2
0.07	1
0.08	2
0.09	8
0.1	39
0.26	1
0.28	1

T2: 6.g. How was the LOQ for 40-3-2 determined (if applicable)?

		Answers	Ratio
Determined from the qPCR analysis for the current sample		21	25.6%
Determined during the in-house validation of the method		43	52.4%
Taken from the EURL GMFF validation report		19	23.2%
By another approach, please explain below		3	3.66%
No Answer		2	2.44%

Explanation on alternative LOQ determination:
Determined from the digital PCR analysis for the current sample
Information about LOQ introduced by the manufacturer (R-Biopharm) of diagnostic kit

T2: 6.h. How did you estimate the measurement uncertainty on the result reported for 40-3-2?

		Answers	Ratio
Uncertainty budget (ISO GUM)		2	2.44%
Uncertainty of the method (in-house validation)		30	36.6%
Known uncertainty of the standard method		8	9.76%
Measurement of replicates (precision)		32	39.0%
From interlaboratory comparison data		4	4.88%
Estimation based on judgement		2	2.44%
In another way, please specify below		7	8.54%
No Answer		2	2.44%

Explanation on alternative determination of measurement uncertainty:
U=S/a √1/p+1/n+(c0-c)2/Sxx
Uncertainty=Coverage Factor (P=95% anf f=n-1) * Standard Deviation / Square-root (Number of Measurements)
MU was estimated according to the Guidance Document on MU for GMO Testing Laboratories JRC ISSN: 1018-5593
We used a calculated k-factor based on the number of repeats
Estimation based on within laboratory reproducibility
95% confidence interval of the results for the current sample
30% of the quantification result
The u was obtain through the estimation of the sd taking into account the repeatability and intermediate precision associated with the test sample. Coverage factor k = 2 was applied then.
Combined Uncertainty (CRM + measurement) following Application note (Lingsinger, 2005, JRC Geel)

Additional comments and suggestions

Event 68416 was not tested due to non-availability of Positive control and reference material.
DNA extraction of T2 has conducted using DNA extraction kits SureFood Prep Basic, etc. (see T1)
The NucleoSpin Food + NucleoSpin gDNA Clean-up was also used for DNA extraction from T1 and T2 samples. The results for 40-3-2 were 25 - 30 % lower than CTAB results:
AOCS 0906-B as calibrant for MON89788 in T2 also

Annex 3: Qualitative performance of the participants

Table A3.1. Performance of the participants for the qualitative identification of GM events in CT-02/17.

The correct answer ("yes") is that the GM event has been detected in the test item.

Lab	40-3-2 detected in T1	40-3-2 detected in T2	Lab	40-3-2 detected in T1	40-3-2 detected in T2
L01	yes	yes	**L45**	yes	yes
L02	yes	yes	**L46**	yes	yes
L03	yes*	yes*	**L47**	yes	yes
L04	yes	yes	**L48**	yes	yes
L05	yes	yes	**L49**	yes*	yes*
L06	yes	yes	**L50**	yes	yes
L07	yes	yes	**L51**	yes	yes
L08	yes	yes	**L52**	yes	yes
L09	yes	yes	**L53**	yes	yes
L10	yes**	Yes**	**L54**	yes	yes
L11	yes	yes	**L55**	yes	yes
L12	yes	yes	**L56**	yes	yes
L13	yes	yes	**L57**	yes	yes
L14	yes	yes	**L58**	yes	yes
L15	yes	yes	**L59**	yes	yes
L16	yes	yes	**L60**	yes	yes
L17	yes	yes	**L61**	yes	yes
L18	yes	yes	**L62**	yes	yes
L19	yes	yes	**L63**	yes	yes
L20	yes	yes	**L66**	yes	yes
L22	yes	yes	**L68**	yes	yes
L23	yes	yes	**L69**	yes	yes
L24	yes	yes	**L70**	yes	yes
L25	yes	yes	**L71**	yes	yes
L26	yes	yes	**L72**	yes	yes
L27	yes	yes	**L73**	T1 was not analysed	yes
L29	yes	yes	**L74**	yes	yes
L30	yes	yes	**L75**	yes	yes
L31	yes	yes	**L76**	yes	yes
L32	yes	yes	**L77**	yes	yes
L33	yes	yes	**L78**	yes	yes
L34	yes	yes	**L79**	yes	yes
L35	yes	yes	**L80**	yes	yes
L36	yes	yes	**L81**	yes	yes
L37	yes	yes	**L82**	yes	yes
L38	yes*	yes*	**L83**	yes	yes
L39	yes	yes	**L84**	yes	yes
L40	yes	yes	**L85**	yes	yes
L41	yes	yes	**L86**	yes	yes
L42	yes	yes	**L87**	yes	yes
L43	T1 was not analysed	yes	**L88**	yes	yes
L44	yes	yes	**L89**	yes	yes

* Although the questionnaire was not returned by the participant, the presence of the event was inferred from the quantitative result reported.

**L10 did not fill in the questionnaire, but reported a "larger than 0.1" result for T1 and T2.

Annex 4: Participants' quantitative performance

The *z* and *ζ* scores of all laboratories are reported in Tables A4.1 and A4.2, and in Figures A4.1 and A4.2, for 40-3-2 soybean in T1 and T2, respectively. For consistency, the reported results are shown with two decimals, the performance scores with one decimal. The *z* scores are displayed in green (satisfactory), orange (questionable) or red cells (unsatisfactory).

Table A4.1. Quantitative results (in m/m %) and performance scores of participants of CT-02/17 for 40-3-2 soybean in chicken feed (T1).

Lab[1]	Category	x_i	U	k^2	Log(x_i)	$u(x_i)$	$u(x_{i\text{-}log})$	*z* score	*ζ* score[3]
L01	NRL/120	0.64	0.08	2.00	-0.19	0.04	0.03	-1.0	-2.8*
L02	NRL/882	0.57	0.02	2.00	-0.24	0.01	0.01	-1.5	-6.7**
L03	Non-NRL	0.73	15.20	2.00	-0.14	7.60	4.52	-0.4	0.0
L04	Non-NRL	0.75			-0.12	0.00	0.00	-0.3	-1.3
L05	NRL/120	0.73	0.07	2.57	-0.14	0.03	0.02	-0.4	-1.5
L06	NRL/882	0.75	0.21	2.00	-0.12	0.11	0.06	-0.3	-0.4
L07	NRL/882	0.65	0.13	2.00	-0.19	0.07	0.04	-0.9	-1.9
L08	NRL/120	0.62	0.23	2.00	-0.21	0.12	0.08	-1.1	-1.3
L09	NRL/882	0.81	0.27	2.00	-0.09	0.14	0.07	0.1	0.1
L12	Non-NRL	1.00	0.51	2.00	0.00	0.26	0.11	1.0	0.9
L13	NRL/882	0.94	0.19	1.73	-0.03	0.11	0.05	0.7	1.3
L14	Non-NRL	0.63			-0.20	0.00	0.00	-1.0	-5.0**
L15	NRL/882	1.10	0.11	2.00	0.04	0.05	0.02	1.4	4.6**
L16	Non-NRL	1.17			0.07	0.00	0.00	1.7	8.0**
L17	NRL/882	0.72	0.10	2.00	-0.14	0.05	0.03	-0.5	-1.2
L18	Non-NRL	0.40			-0.40	0.00	0.00	**-3.0**	-14.5**
L19	Non-NRL	1.15	0.63	1.73	0.06	0.36	0.14	1.6	1.1
L20	NRL/120	1.08	0.32	2.00	0.03	0.16	0.06	1.3	1.9
L22	Non-NRL	0.51	0.30	2.23	-0.29	0.13	0.11	-2.0	-1.7
L23	Non-NRL	0.47	0.09	2.00	-0.33	0.05	0.04	**-2.3**	-5.0**
L24	NRL/882	0.72	0.19	2.00	-0.14	0.10	0.06	-0.5	-0.7
L25	Non-NRL	1.20			0.08	0.00	0.00	1.8	8.5**
L26	NRL/882	0.23	0.09	2.00	-0.64	0.05	0.08	**-5.4**	-6.2**
L27	Non-NRL	0.98	0.15	2.00	-0.01	0.08	0.03	0.9	2.3*
L29	NRL/120	0.90	0.08	1.73	-0.05	0.05	0.02	0.5	1.7
L30	NRL/882	0.45	0.13	2.00	-0.35	0.07	0.06	**-2.5**	-3.8**
L31	NRL/882	0.80	0.38	2.23	-0.10	0.17	0.09	0.0	0.0
L32	Non-NRL	0.76	0.39	2.00	-0.12	0.20	0.11	-0.2	-0.2
L33	NRL-120	0.57	0.14	2.00	-0.24	0.07	0.05	-1.5	-2.6*
L34	NRL/120	0.61	0.16	2.00	-0.21	0.08	0.06	-1.2	-1.9
L35	NRL/882	0.97	0.27	2.00	-0.01	0.14	0.06	0.8	1.3
L36	Non-NRL	0.90	0.30	2.00	-0.05	0.15	0.07	0.5	0.7
L37	NRL/120	0.65	0.26	2.00	-0.19	0.13	0.09	-0.9	-1.0
L38	NRL/120	0.93	0.14	2.37	-0.03	0.06	0.03	0.7	1.9
L39	Non-NRL	1.12			0.05	0.00	0.00	1.5	7.1**
L40	NRL/882	0.53	0.08	2.00	-0.28	0.04	0.03	-1.8	-4.6**
L41	NRL/120	0.80	0.03	2.00	-0.10	0.02	0.01	0.0	0.0
L42	NRL/882	1.04	0.31	2.00	0.02	0.16	0.06	1.1	1.7
L44	NRL/120	0.84	0.07	2.57	-0.08	0.03	0.01	0.2	0.9
L45	NRL/882	0.65	0.18	2.00	-0.19	0.09	0.06	-0.9	-1.4
L46	NRL/882	0.81	0.18	2.00	-0.09	0.09	0.05	0.1	0.1
L47	NRL/882	1.41	0.27	1.73	0.15	0.16	0.05	**2.5**	4.7**
L48	NRL/882	0.59	0.28	2.00	-0.23	0.14	0.10	-1.3	-1.3
L49	Non-NRL	0.89	0.31	2.00	-0.05	0.16	0.08	0.5	0.6
L50	NRL/120	0.73	0.05	2.00	-0.14	0.03	0.01	-0.4	-1.5
L51	Non-NRL	0.77	0.49	2.00	-0.11	0.25	0.14	-0.2	-0.1
L52	Non-NRL	0.91	0.12	2.00	-0.04	0.06	0.03	0.6	1.6
L53	Non-NRL	0.43	0.81	2.00	-0.37	0.41	0.41	**-2.7**	-0.7
L54	NRL/882	0.74	0.35	2.00	-0.13	0.18	0.10	-0.3	-0.3
L55	Non-NRL	0.76	0.19	1.73	-0.12	0.11	0.06	-0.2	-0.3

Lab[1]	Category	x_i	U	k[2]	Log(x_i)	$u(x_i)$	$u(x_{i\text{-}log})$	z score	ζ score[3]
L56	NRL/882	1.15	0.22	1.73	0.06	0.13	0.05	1.6	3.0**
L57	NRL/120	0.75	0.20	2.00	-0.12	0.10	0.06	-0.3	-0.4
L58	NRL/120	0.89	0.16	2.00	-0.05	0.08	0.04	0.5	1.1
L59	NRL/882	1.37	0.38	2.00	0.14	0.19	0.06	**2.3**	3.7**
L60	NRL/882	0.68	0.17	2.00	-0.17	0.09	0.05	-0.7	-1.2
L61	Non-NRL	0.65			-0.19	0.00	0.00	-0.9	-4.3**
L62	NRL/120	1.30	0.37	2.37	0.11	0.16	0.05	**2.1**	3.8**
L63	Non-NRL	0.88	0.06	2.00	-0.06	0.03	0.01	0.4	1.6
L64	NRL/882	0.81	0.24	1.73	-0.09	0.14	0.07	0.1	0.1
L65	Non-NRL	1.16	0.65	2.00	0.06	0.33	0.12	1.6	1.3
L66	NRL/882	0.48	0.24	2.00	-0.32	0.12	0.11	**-2.2**	-2.0
L68	NRL/882	1.39	0.15	2.00	0.14	0.08	0.02	**2.4**	7.7**
L69	Non-NRL	0.10	0.04	2.00	-1.01	0.02	0.08	**-9.1**	-10.5**
L70	Non-NRL	0.57	0.10	2.00	-0.24	0.05	0.04	-1.5	-3.4**
L72	NRL/882	1.08	0.32	2.00	0.03	0.16	0.07	1.3	1.9
L74	NRL/882	0.75	0.19	2.00	-0.12	0.10	0.05	-0.3	-0.5
L75	Non-NRL	8.62			0.94	0.00	0.00	**10.3**	49.9**
L76	NRL/882	0.99	0.38	2.00	0.00	0.19	0.08	0.9	1.1
L77	NRL/120	0.75	0.09	2.26	-0.12	0.04	0.02	-0.3	-0.9
L78	NRL/120	0.59	0.06	3.00	-0.23	0.02	0.01	-1.3	-5.2**
L79	Non-NRL	0.46			-0.34	0.00	0.00	**-2.4**	-11.6**
L80	NRL/120	0.71	0.24	2.00	-0.15	0.12	0.07	-0.5	-0.7
L81	NRL/882	0.71	0.28	2.00	-0.15	0.14	0.09	-0.5	-0.6
L82	NRL/120	0.92	0.15	2.00	-0.04	0.08	0.04	0.6	1.5
L83	NRL/120	1.14	0.13	1.73	0.06	0.08	0.03	1.5	4.4**
L84	Non-NRL	0.71	0.30	2.00	-0.15	0.15	0.09	-0.5	-0.5
L85	NRL/882	0.74	0.31	2.01	-0.13	0.15	0.09	-0.3	-0.4
L86	Non-NRL	0.66	0.22	2.00	-0.18	0.11	0.07	-0.8	-1.1
L87	NRL/882	0.85	0.15	2.00	-0.07	0.08	0.04	0.3	0.6
L88	NRL-120	**<0.04**[4]							**
L89	NRL/882	0.79	0.22	2.00	-0.10	0.11	0.06	-0.1	-0.1

[1] One NRL/882 (L73) did not report a quantitative result for 40-3-2 in T1 because T1 is out of scope of the laboratory.
[2] If the k factor was not reported by the laboratory, a value of 1.73 was assigned ($\sqrt{3}$) for calculation of the ζ score.
[3] The ζ scores are provided for information only, with * indicative of a questionable score and ** of an unsatisfactory score.
[4] The "less than value" reported by the laboratory is considered unsatisfactory.

Table A4.2. Quantitative results (in m/m %) and performance scores of participants of CT-02/17 for 40-3-2 soybean in soybean flour (T2).

Lab[1]	Category	x_i	U	k^2	Log(x_i)	$u(x_i)$	$u(x_{i-log})$	z score	ζ score[3]
L01	NRL/120	0.71	0.07	2.00	-0.15	0.04	0.02	-0.3	-1.2
L02	NRL/882	0.81	0.03	2.00	-0.09	0.02	0.01	0.3	1.9
L03	Non-NRL	0.88	15.20	2.00	-0.06	7.60	3.75	0.6	0.0
L04	Non-NRL	0.89			-0.05	0.00	0.00	0.7	5.6**
L05	NRL/120	0.75	0.05	2.57	-0.12	0.02	0.01	-0.1	-0.4
L06	NRL/882	0.80	0.22	2.00	-0.10	0.11	0.06	0.2	0.4
L07	NRL/882	0.76	0.09	2.00	-0.12	0.05	0.03	0.0	0.0
L08	NRL/120	0.42	0.16	2.00	-0.38	0.08	0.08	**-2.6**	-3.1**
L09	NRL/882	0.81	0.27	2.00	-0.09	0.14	0.07	0.3	0.4
L12	Non-NRL	0.76	0.39	2.00	-0.12	0.20	0.11	0.0	0.0
L13	NRL/882	0.69	0.11	1.73	-0.16	0.06	0.04	-0.4	-1.0
L14	Non-NRL	0.75			-0.12	0.00	0.00	-0.1	-0.5
L15	NRL/882	0.84	0.11	2.00	-0.08	0.05	0.03	0.4	1.4
L16	Non-NRL	0.88			-0.06	0.00	0.00	0.6	5.2**
L17	NRL/882	0.75	0.10	2.00	-0.12	0.05	0.03	-0.1	-0.2
L18	Non-NRL	0.34			-0.47	0.00	0.00	**-3.5**	-28.7**
L19	Non-NRL	0.89	0.16	1.73	-0.05	0.09	0.05	0.7	1.5
L20	NRL/120	1.04	0.31	2.00	0.02	0.16	0.06	1.4	2.1*
L22	Non-NRL	0.81	0.26	2.23	-0.09	0.12	0.06	0.3	0.4
L23	Non-NRL	0.64	0.12	2.00	-0.19	0.06	0.04	-0.8	-1.8
L24	NRL/882	0.79	0.21	2.00	-0.10	0.11	0.06	0.2	0.3
L25	Non-NRL	0.85			-0.07	0.00	0.00	0.5	3.9**
L26	NRL/882	1.17	0.39	2.00	0.07	0.20	0.07	1.9	2.5*
L27	Non-NRL	1.18	0.18	2.00	0.07	0.09	0.03	1.9	5.4**
L29	NRL/120	0.78	0.04	1.73	-0.11	0.02	0.01	0.1	0.6
L30	NRL/882	0.69	0.20	2.00	-0.16	0.10	0.06	-0.4	-0.7
L31	NRL/882	0.96	0.77	2.33	-0.02	0.33	0.15	1.0	0.7
L32	Non-NRL	0.90	0.39	2.00	-0.05	0.20	0.09	0.7	0.8
L33	NRL-120	0.78	0.04	2.00	-0.11	0.02	0.01	0.1	0.6
L34	NRL/120	0.73	0.14	2.00	-0.14	0.07	0.04	-0.2	-0.4
L35	NRL/882	0.95	0.27	2.00	-0.02	0.14	0.06	1.0	1.5
L36	Non-NRL	0.77	0.03	2.00	-0.11	0.02	0.01	0.1	0.3
L37	NRL/120	0.75	0.10	2.00	-0.12	0.05	0.03	-0.1	-0.2
L38	NRL/120	1.19	0.08	2.37	0.08	0.03	0.01	1.9	11.2**
L39	Non-NRL	0.62			-0.21	0.00	0.00	-0.9	-7.3**
L40	NRL/882	0.45	0.08	2.00	-0.35	0.04	0.04	**-2.3**	-5.6**
L41	NRL/120	0.87	0.02	2.00	-0.06	0.01	0.00	0.6	4.4**
L42	NRL/882	0.68	0.20	2.00	-0.17	0.10	0.06	-0.5	-0.8
L43	Non-NRL	0.65			-0.19	0.00	0.00	-0.7	-5.6**
L44	NRL/120	0.74	0.06	2.20	-0.13	0.03	0.02	-0.1	-0.6
L45	NRL/882	0.73	0.21	2.00	-0.14	0.11	0.06	-0.2	-0.3
L46	NRL/882	0.81	0.18	2.00	-0.09	0.09	0.05	0.3	0.5
L47	NRL/882	0.62	0.12	1.73	-0.21	0.07	0.05	-0.9	-1.8
L48	NRL/882	0.74	0.35	1.73	-0.13	0.20	0.12	-0.1	-0.1
L49	Non-NRL	0.73	0.26	2.00	-0.14	0.13	0.08	-0.2	-0.2
L50	NRL/120	0.81	0.07	2.00	-0.09	0.04	0.02	0.3	1.2
L51	Non-NRL	0.68	0.44	2.00	-0.17	0.22	0.14	-0.5	-0.3
L52	Non-NRL	0.73	0.11	2.00	-0.14	0.06	0.03	-0.2	-0.5
L53	Non-NRL	0.58	0.81	2.00	-0.24	0.41	0.30	-1.2	-0.4
L54	NRL/882	0.73	0.26	2.00	-0.14	0.13	0.08	-0.2	-0.2
L55	Non-NRL	0.72	0.18	1.73	-0.14	0.10	0.06	-0.2	-0.4
L56	NRL/882	0.66	0.18	1.73	-0.18	0.10	0.07	-0.6	-0.9
L57	NRL/120	0.60	0.20	2.00	-0.22	0.10	0.07	-1.0	-1.4
L58	NRL/120	0.67	0.11	2.00	-0.17	0.06	0.04	-0.6	-1.5
L59	NRL/882	0.76	0.22	2.00	-0.12	0.11	0.06	0.0	0.0
L60	NRL/882	0.80	0.20	2.00	-0.10	0.10	0.05	0.2	0.4
L61	Non-NRL	0.50			-0.30	0.00	0.00	-1.8	-15.0**

Lab[1]	Category	x_i	U	k^2	Log(x_i)	$u(x_i)$	$u(x_{i-log})$	z score	ζ score[3]
L62	NRL/120	0.75	0.12	2.45	-0.12	0.05	0.03	-0.1	-0.2
L63	Non-NRL	0.75	0.04	2.00	-0.12	0.02	0.01	-0.1	-0.4
L64	NRL/882	0.78	0.23	1.73	-0.11	0.13	0.07	0.1	0.1
L65	Non-NRL	0.51	0.23	2.00	-0.29	0.12	0.10	-1.7	-1.8
L66	NRL/882	0.73	0.24	2.00	-0.14	0.12	0.07	-0.2	-0.2
L68	NRL/882	0.91	0.11	2.00	-0.04	0.06	0.03	0.8	2.7*
L69	Non-NRL	0.92	0.35	2.00	-0.04	0.18	0.08	0.8	1.0
L70	Non-NRL	0.50	0.09	2.00	-0.30	0.05	0.04	-1.8	-4.5**
L72	NRL/882	0.92	0.28	2.00	-0.04	0.14	0.07	0.8	1.2
L73	NRL/882	0.92	0.30	2.00	-0.04	0.15	0.07	0.8	1.1
L74	NRL/882	0.64	0.13	2.00	-0.19	0.07	0.04	-0.8	-1.6
L75	Non-NRL	6.44			0.81	0.00	0.00	**9.3**	76.0**
L76	NRL/882	0.56	0.99	2.00	-0.25	0.50	0.38	-1.3	-0.3
L77	NRL/120	0.54	0.06	2.26	-0.27	0.03	0.02	-1.5	-6.1**
L78	NRL/120	0.65	0.01	3.00	-0.19	0.00	0.00	-0.7	-5.5**
L79	Non-NRL	0.71			-0.15	0.00	0.00	-0.3	-2.5*
L80	NRL/120	0.95	0.30	2.00	-0.02	0.15	0.07	1.0	1.4
L81	NRL/882	0.77	0.32	2.00	-0.11	0.16	0.09	0.1	0.1
L82	NRL/120	1.02	0.32	2.00	0.01	0.16	0.07	1.3	1.8
L83	NRL/120	0.84	0.05	1.73	-0.08	0.03	0.01	0.4	2.2*
L84	Non-NRL	0.45	0.30	2.00	-0.35	0.15	0.14	**-2.3**	-1.6
L85	NRL/882	0.82	0.15	2.12	-0.09	0.07	0.04	0.3	0.8
L86	Non-NRL	0.73	0.24	2.00	-0.14	0.12	0.07	-0.2	-0.2
L87	NRL/882	0.77	0.12	2.00	-0.11	0.06	0.03	0.1	0.1
L88	NRL/120	0.65	0.20	2.00	-0.19	0.10	0.07	-0.7	-1.0
L89	NRL/882	0.77	0.21	2.00	-0.11	0.11	0.06	0.1	0.1

[1] All participating NRL/882 reported a quantitative result for 40-3-2 soybean in T2.
[2] If the k factor was not reported by the laboratory, a value of 1.73 was assigned ($\sqrt{3}$) for calculation of the ζ score.
[3] The ζ scores are provided for information only, with * indicative of a questionable score and ** of an unsatisfactory score.

Figure A4.1. Laboratory results for soybean event 40-3-2 in test item 1 and kernel density distribution (insert).

The horizontal full line shows the assigned value (on the log scale), the dashed black lines represent the expanded measurement uncertainty of the assigned value, and the wider interval (dashed red lines) represents the limits of satisfaction ($|z| \leq 2.0$). Laboratory results are shown with the expanded measurement uncertainties (when reported); the expanded measurement uncertainty of L03 is out of scale.

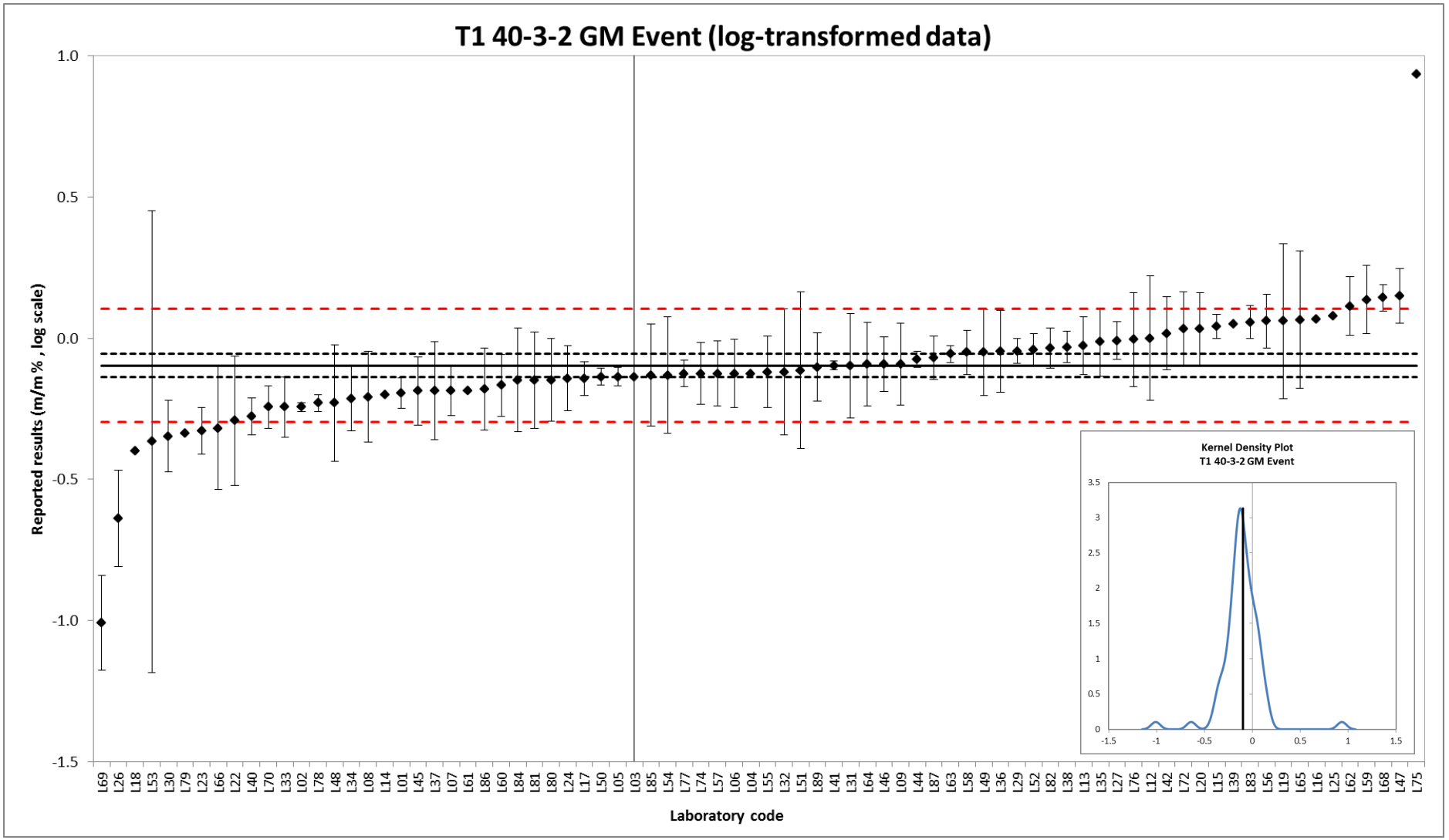
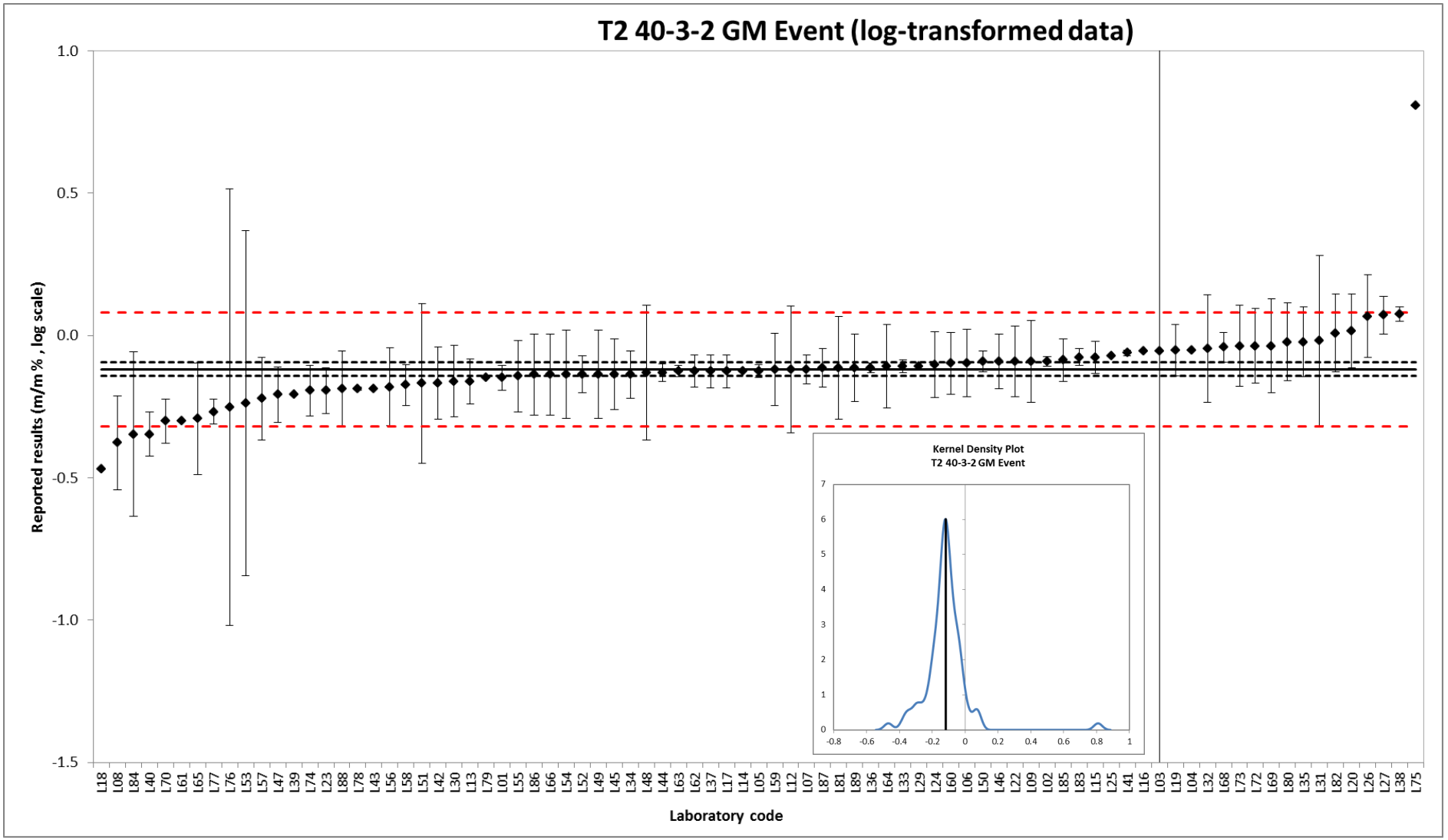


Figure A4.2. Laboratory results for soybean event 40-3-2 in test item 2 and kernel density distribution (insert).

The horizontal full line shows the assigned value (on the log scale), the dashed black lines represent the expanded measurement uncertainty of the assigned value, and the wider interval (dashed red lines) represents the limits of satisfaction ($|z| \leq 2.0$). Laboratory results are shown with the expanded measurement uncertainties (when reported); the expanded measurement uncertainty of L03 is out of scale.



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