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Determination of GM Soybean 44406 in Soya Milk Powder and GM Maize VCO-1981 in Maize Flour

Proficiency test report
EURL-GMFF-CT-01/17

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Contents

Quality assurance

The European Union Reference Laboratory for GM Food and Feed (EURL GMFF), hosted by the Joint Research Centre (JRC) in Ispra (Italy), is accredited according to ISO/IEC 17043:2010 (accreditation number: ACCREDIA 0012) for the organisation of proficiency tests (here called comparative tests or CT).

The EURL GMFF is also accredited according to ISO/IEC 17025:2005 (accreditation number: ACCREDIA 1172) for testing methods on food and feed (flexible scope) for GMOs (DNA extraction, detection, identification and quantification by PCR).

The activities described in this report for the JRC-Geel (Belgium) have been performed under a quality management system accredited according to ISO/IEC 17025:2005 (accreditation number: BELAC 268) for the testing of plant material (flexible scope) for GM content (DNA extraction, real-time PCR), and according to ISO Guide 34:2009 for the production of certified reference materials.

No 0012

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 comparative testing round will receive a unique lab code that will be used throughout this report.

List of abbreviations

CT	Comparative testing (= proficiency testing or PT)
EURL	European Union Reference Laboratory
GMFF	Genetically modified food and feed
kbp	Kilo (1000) basepairs
m/m %	Mass per mass percentage
MW	Molecular weight
N.A.	Not applicable
NRL	National Reference Laboratory in line with Regulation (EC) No 882/2004 (NRL/882) on official controls in food and feed or with Regulation (EU) No 120/2014 (NRL/120), cooperating with the EURL GMFF on method validation
OCL	Official Control Laboratory
qPCR	Quantitative (real-time) Polymerase Chain Reaction
SD	Standard deviation
σ_{pt}	Standard deviation for proficiency testing
U/u	Expanded/standard measurement uncertainty
z and *ζ*	Variables used to express the performance of a laboratory

Executive summary

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

Two test items were distributed: a soya milk powder spiked with soybean GM event DAS-444Ø6-6 (Test Item 1, T1) and a maize flour containing maize event VCO-Ø1981-5 (Test Item 2, T2). Participants were required to screen T1 and T2 for the presence of three GM soybean events and three GM maize events, respectively, and to quantify those events identified. The results had to be reported in GM mass/mass %.

Eighty-three participants from 38 countries participated to this CT round, including 55 NRLs, of which 33 are designated in line with Regulation (EC) No 882/2004 (NRL/882) and 22 are nominated in Regulation (EU) No 120/2014 to support the EURL GMFF on method validation (NRL/120).

The qualitative results, i.e. the correct identification of the GM events, were evaluated and scored as correct or incorrect. The assigned value for the 44406 soybean mass fraction in the soy milk material was derived as the robust mean of the data provided by NRLs, while for VCO-1981, the certified value was set as the assigned value. *z and ζ* scores were calculated to assess laboratory performance.

The results reported indicate that all NRLs identified the correct GM events in both test items and most of the quantitative results were satisfactorily. Five laboratories, including one NRL/882, obtained an unsatisfactory *z* score for the quantification of 44406 soybean in soya milk powder. All *z* scores for VCO-1981 maize content in maize flour were satisfactory. A total of 19 and 7 unsatisfactory *ζ* scores were obtained for events 44406 and VCO-1981, respectively. At least for a number of laboratories this was the due to an underestimation or overestimation of the measurement uncertainty or a failure to report it.

A root-cause analysis will be requested from NRLs having reported unsatisfactory results in this CT round 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; a part of these NRL/120 are also NRL/882). The Member States of the European Union may also appoint other laboratories (non-NRLs) for performing the official controls on food and feed.

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

The EURL GMFF is tasked with the organisation of proficiency tests (here called comparative tests or CT in line with the legislation[2]) to foster the correct application of the analytical methods available for the official controls[2]. 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 CT round organised by the EURL GMFF in 2017 (CT 01/17). Participation in such CT rounds is mandatory for NRL/882, recommended for NRL/120, and open to official control laboratories within or outside the EU.

2 Test items

The test items used in this CT round 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 soya milk powder (Now Foods, Real Food, USDA Organic Soy Milk) and the ERM-BF436b containing the DAS-44406-6 event (hereafter named 44406) as spiked material (Table 1).

For the soya milk powder and the ERM-BF436b powder, a residual water mass fraction of 22.6 ± 3.2 g/kg and 45.5 ± 6.4 g/kg, respectively, was measured by volumetric Karl Fischer titration (758 KFD Titrino, Metrohm, Herisau, CH) with the expanded uncertainty calculated with a coverage factor of k = 2. Both powders were sufficiently dry to perform the dry mixing and did not require an additional drying step.

The particle volumes for both powders were measured based on laser diffraction patterns (PSA, Sympatec, Clausthal-Zellerfeld, DE) and were compared. The mean particle diameters (N = 1, n = 5), calculated by the PSA software, were 74.1 µm (SD = 0.4 µm) for the soya milk powder and 93 µm (SD = 9 µm) for the ERM-BF436b powder. It was concluded that the particle volume fractions of both powders were sufficiently similar to allow the processing of mixtures without introducing an additional bias as the result of a different DNA extractability.

The amount and the quality of the DNA extracted from the soya milk powder and the GM spiking material were verified by UV spectrometry, fluorometry and gel electrophoresis. Four different extraction methods were tested: a DNeasy Plant Mini kit, a CTAB method[6], and two other CTAB methods optimised for soybean with and without purification step by Genomic-tip 20/G (Qiagen, Hilden, Germany). Sample intakes of 50, 100 and 200 mg were tested. The DNA extraction method offering the highest yield with an optimal $Abs_{260/280}$ ratio was the JRC-GEEL in-house CTAB extraction method without extra column purification (Table 1). DNA extracted with the in-house CTAB methods (referred to as C and D in Table 1) were tested between 40 ng/µL to 0.02 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 between 96 and 98 % with a coefficient of determination (R^2) between 0.99 and 1, confirming the absence of significant amounts of PCR inhibitors in the extracts.

The CTAB method (C) was chosen for all further analyses because it yielded a sufficient amount of DNA of PCR grade quality from both base materials.

However, the amount of DNA extracted from the soya milk powder was seriously underestimated when measured by UV fluorometry (PicoGreen). Upon agarose gel electrophoresis, this DNA showed a high level of fragmentation (smear from ± 25 to 1 kbp), while the DNA extracted from the spiking material migrated as a high molecular weight band (above 25 kbp). The precise industrial process that has been used to transform the soybean seeds into a soya milk powder used in this CT is unknown. However, instant soya milk powders are often produced by a succession of evaporations or ultrafiltration, spray drying and fluidised bed agglomeration[7]. Some of those processes may be disruptive and generate a mixture of small single-stranded and double-stranded (ds) DNA. As the PicoGreen assay only measures dsDNA and as the final GM percentage is affected by the amount of DNA that can be extracted and amplified by PCR, it was decided to estimate the amount of DNA per mg soya milk powder on the basis of the amount of amplifiable lectin fragments. That amount was determined by qPCR with a lectin assay using DNA from the spiking material as calibrant.

The yield of amplifiable DNA per mg soya milk powder and the yield of DNA measured by PicoGreen for the spiking material were both taken into account to calculate the amount of spike material and soya milk powder to be mixed to obtain a target value of 0.6 m/m %.

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

Characteristic	Soya Milk	44406 Soybean
Type of base material	Instant soya milk powder	CRM
Origin	Now Foods, Real Food, USDA Organic Soy Milk	ERM-BF436b[8]
Grinding method	None, used as such	Cryo-grinding vibrating mill
Mixing method	DynaMIX CM200 (WAB, Basel, CH)	
Water content in m/m %, mean ± *U* (*k* = 2, *n* = 3)	45.5 ± 6.4	22.6 ± 3.2
Particle diameter in µm ± SD (*n* = 5)	74.1± 0.4	93 ± 9
DNA yield in ng/mg[1], mean ± SD (*n* = 2 - 3)	A: 20.4 ± 2.8 B: 5.0 ± 2.5 C: 65.5 ± 2.5 D: 33.1 ± 6.1	A: 10.3 ± 4.0 B: 1.8 ± 0.7 C: 72.0 ± 3.8 D: 70.3 ± 0.2
Genetic elements detected with screening pre-spotted plates	Hmg, Lec, P35S (Cq 33.3), tNOS (Cq 39.91), CTP2_EPSPS (Cq 34.02)	Lec, PAT
GM soybean events detected with event-specific pre-spotted plates[2]	GTS 40-3-2 (Cq 33.75), MON89788 (Cq 33.32)	none
Mass used to prepare T1 (g)		
Intermediate mixture T01	1700	100
Final test item T1	1600	100 of T01
Nominal target GM mass fraction in T1 (m/m %) measured by qPCR	NA	0.6 m/m %

[1] Results reported here for a sample intake of 200 mg. A: CTAB method[6], B: DNeasy Plant Mini kit[6], C: in-house validated CTAB method for soybean (JRC-GEEL), D: in-house validated CTAB method for soybean, omitting the Genomic-tip 20/G purification (JRC-GEEL). The DNA yield values for the soya milk were determined by qPCR, whereas the yield for the spiking material 44406 was measured by fluorometry.
[2] An all-species event-specific pre-spotted plate (PSP) was used for all tests; the PSP version used does not contain the 44406 method.
NA: not applicable; SD: standard deviation; k: coverage factor; *U*: expanded uncertainty.

The presence of unexpected GM events in the base materials and in a pilot mixture was tested by using the screening[9] and GM soybean event-specific pre-spotted plates[10]. The organic soya milk powder (labelled "non GMO") contained traces of p35S, tNOS and CTP2-EPSPS, GTS 40-3-2 and MON89788 at a level estimated around 0.01 m/m %.

The final test items were prepared gravimetrically in accordance with ISO Guide 34[11] as follows:

- The mass of the GM ingredient to add (44406 soybean) was calculated taking into account the amount of DNA that could be extracted and amplified from both materials (Table 1).
- The compound sample T1 was mixed in a DynaMIX CM200 (WAB, Basel, CH) for 1 h to improve equal distribution of the different types of soya tissues.
- 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 prevent accidental opening during storage and transport.
- A total of 336 vials containing each at least 5 g of flour were then labelled with a sample number and the description "Sample T1 (Food, soya milk)".
- 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 both GM events (*p*-value > 0.05; 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 covered by this CT (Annex 1).

JRC-Ispra tested the T1 material and confirmed the results obtained by JRC-Geel. The average measured mass fraction for event 44406 in T1 (0.57 ± 0.09 (k=2) m/m %; n = 104) was in agreement with the expected nominal value.

2.2 Test item 2

The T2 test item was a certified reference material (Table 2). The bottles of T2 were re-labelled with a unique sample number as "Sample T2 (Feed, maize)".

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 CT (Annex 1).

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

Characteristic	**Soybean feed**
Type of base material	CRM
Origin	ERM-BF438d[12] containing 10.0 ± 0.8 g/kg VCO-Ø1981-5 maize, produced in 2015 by JRC-Geel; prepared from a hemizygous GMO with the GM event contributed from the male parent during hybrid production

3 Instructions to the participants

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

Test Item 1: "Food, soya milk"

- Screen for the presence of the following three GM soybean event(s): 44406, CV127, MON87708;
- Quantify the GM soybean event(s) detected.

Test Item 2: "Feed, maize"

- Screen for the presence of the following three GM maize events: MON810, NK603, VCO-1981;
- Quantify the GM maize event(s) detected.

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 reminded of the general rule that results obtained using a calibrant certified for GM mass fraction (*i.e.* a matrix CRM certified in [x] g/kg) can directly be expressed in m/m %, while results obtained using a calibrant certified for DNA copy number ratio (*e.g.* a plasmid containing both the GM and reference gene target or some matrix CRMs) need to be converted into m/m %, using a conversion factor[13,14].

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

4 Results

4.1 Participation to CT round 01/17

On 23 February 2017, 201 laboratories were invited to participate in the CT round EURL-GMFF-CT-01/17 and 88 laboratories registered for it and received a random unique lab code (L01 to L88). Eighty-three laboratories from 38 countries returned results within the reporting deadline. Five laboratories did not submit any results, one of which (L77, non-NRL) had not received the samples from customs in time and one NRL/882 (L65) had a problem with ordering the reagents in time; for the remaining 3 non-NRLs the reason for not participating is not known.

Table 3 shows an overview of the participation to this CT round.

Table 3. Invitation and participation to the comparative testing round CT 01/17.

Characteristic of the CT round	Result
Date of invitation	23 February 2017
Number of invited laboratories	201
Number of registered laboratories	88
Date of shipment of samples	29 March 2017
Deadline for result submission	12 May 2017
Registered laboratories that failed to submit their data	5
Number of participating laboratories	83

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 24 EU Member States (many of these are also NRL/120). In addition, Ireland is delegating its NRL/882 tasks to one of the CT participants (Rikilt, NL). Estonia, Greece and Malta were not represented in this CT round.
- Twenty-two NRLs nominated under Regulation (EU) No 120/2014 (NRL/120) who are not at the same time NRLs under Regulation (EC) No 882/2004.
- Twenty-eight official control laboratories, but not NRLs nominated under either of the Regulations mentioned above. This category includes 10 EU official control laboratories (OCLs) and 18 OCLs from non-EU countries, including Serbia and Switzerland.

Table 4. Overview of participants to CT 01/17 by country and category.

Country	Participants	NRL/882[1]	NRL/120	Non-NRL
AUSTRIA	2	2		
BELGIUM	4	3		1
BRAZIL	1			1
BULGARIA	3	1		2
COLOMBIA	1			1
CROATIA	2	1		1
CYPRUS	1	1		
CZECH REPUBLIC	1	1		
DENMARK	1	1		
FINLAND	2	1	1	
FRANCE	3	3		
GERMANY	17	1	15	1
HUNGARY	2	1		1
ITALY	5	1	2	2
LATVIA	1	1		
LITHUANIA	1	1		
LUXEMBOURG	1	1		
MALAYSIA	1			1
MEXICO	1			1
NETHERLANDS	2	1	1	
POLAND	5	3	1	1
PORTUGAL	1	1		
ROMANIA	2	1		1
SERBIA	1			1
SINGAPORE	1			1
SLOVAKIA	2	2		
SLOVENIA	1	1		
SOUTH AFRICA	1			1
SPAIN	2	2		
SWEDEN	1	1		
SWITZERLAND	2			2
THAILAND	1			1
TUNISIA	1			1
TURKEY	2			2
UKRAINE	1			1
UNITED KINGDOM	3	1	2	
UNITED STATES	1			1
VIETNAM	3			3
Total	**83**	**33**	**22**	**28**

[1] No NRL/882 from Estonia, Greece and Malta participated to this CT round.

4.2 Information on the testing provided in the questionnaire

Participants were asked to fill in an online questionnaire (through EUSurvey) on their testing methodology for T1 and T2, consisting of a number of mostly multiple-choice questions. A total of 75 laboratories completed the questionnaire, including 30 out of 33 NRL/882, 17 out of 22 NRL/120 and all 28 non-NRLs (L11, L18, L24, L32, L44, L46, L49 and L58 did not fill in the questionnaire). Not all laboratories, however, provided an answer to all questions that were relevant for their analysis.

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 01/17.

Question (and Question number)	Test Item 1 - 44406	Test Item 2 – VCO-1981
Test item analysed	Yes (69[1]), No (6)	Yes (75), No (0)
Reason for lack of analysis (Q1)	Matrix out of scope (2), methods not validated (2)	-
DNA extraction method (Q2)	CTAB (30), NucleoSpin Food (12)	CTAB (37), NucleoSpin Food (10)
Additional DNA purification method (Q3)	None (45), Ethanol (10)	None (47), Ethanol (11)
Number of replicates (Q4)	2 (44), 4 (10)	2 (49), 4 (12)
Approach to test for PCR inhibition (Q5)	OD ratios (36), delta Cq or GM % between two dilutions (31)	OD ratios (33), delta Cq or GM % between two dilutions (33)
Reason for not testing all events (Q9)	Not applicable (53), reagents not available (11)	Not applicable (50), reagents not available (7)
Approach used (Q6a/8a)	Standard curves (47), delta Cq (7)	Standard curves (44), delta Cq (8)
Calibrant used (Q6b/8b)	CRM JRC-Geel in m/m % (52), no calibrant (2)	CRM JRC-Geel in m/m % (49), other RM (1)
Taxon-specific endogenous gene (Q6c/8c)	*lec*-74 bp (49), *lec*-81 bp (2)	*hmg* (31), aldolase (15)
Unit of measurement and data expression (Q6d/8d)	Mass (37), copies=mass CRM (16)	Mass (37), copies=mass CRM (11)
Amount of DNA (Q6e/8e)	100 ng (22), 200 ng (16)	200 ng (18), 100 ng (15)
LOQ (Q6f/8f)	0.1 (31), <0.1 (21)	0.1 (24), <0.1 (19)
LOQ determination (Q6g/8g)	EURL validation (23), current analysis (19)	EURL validation (21), current analysis (20)
Uncertainty determination (Q6h/8h)	Precision of replicates (29), in-house validation (12)	Precision of replicates (26), in-house validation (10)

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

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. In the majority of laboratories two replicate DNA extracts were analysed. A minority of laboratories performed a PCR inhibition run on 3 or 4 DNA dilutions with a reference gene before analysis. Most laboratories only checked the quality of the DNA extracts by verifying the OD ratios, and/or running two dilutions.

For the quantitative analysis, the most common approach used was based on two standard curves, however, 7 and 8 laboratories applied the delta Cq approach for 44406 soybean and VCO-1981 maize, respectively. Two laboratories (L13 and L39) mentioned the use of digital PCR for 44406 soybean quantification (note: L13 received an unsatisfactory *z* score for this result). The CRM from JRC-Geel (former IRMM) were used by all laboratories that filled in the question, except one NRL/120 (L69) who used a non-certified reference material (RM) for both GM assays. *Lec* was used as taxon-specific reference gene by most laboratories for soybean. For maize, most laboratories used *hmg*, but aldolase was used by 15 laboratories. The majority of laboratories performed their measurements in the same unit as the certified value of the calibrant used (m/m %), whereas 16 (T1) and 11 (T2) laboratories used DNA copies in their calculation sheets, but assumed that 10 % in m/m % equalled 10 % in copy/copy (see further below). The LOQ reported was either taken from the EURL GMFF validation report or determined from the analysis results for the event. In most cases a LOQ of 0.1 m/m % or lower was reported. The measurement uncertainty was mostly estimated from the precision of the analysis results.

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	44406	45 (+8)	0	0	2
		CV127	0	45	0	
		MON87708	0	44	1	
	T2	MON810	1	46	0	0
		NK603	1	46	0	
		VCO-1981	45 (+6)	0	2 (+2)	
Non-NRLs	T1	44406	14 (+2)	0	8	4
		CV127	1	19	4	
		MON87708	1	16	7	
	T2	MON810	1	27	0	0
		NK603	1	27	0	
		VCO-1981	9 (+1)	0	17 (+1)	

One NRL/882 reported that T1 was out of the scope of the laboratory, as agreed between the NRLs within the Member State and communicated to the EURL GMFF; in this case a sister NRL/882 in the same Member State provided results for this sample. Also one NRL/120 reported that the T1 matrix was out of their scope of analysis.

All 53 NRLs who had tested T1 identified the 44406 event in T1 and found CV127 and MON87708 absent. For T2, 51 NRLs identified event VCO-1981, but 4 NRLs (including two NRL/882) had not tested this event. One NRL (L41) also detected MON810 and NK603 in T2 at low concentrations. Despite 44406 and VCO-1981 being rather recent GM events that have not yet been authorised in the EU, the results show that most EU NRLs are able to correctly identify them in a food or feed matrix.

The results of the non-NRLs were also largely satisfactorily, however, a larger proportion of laboratories did not test for event 44406 and particularly for event VCO-1981.

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

Of the 83 laboratories that participated to this CT round, the number of participants that submitted event-specific quantitative data for each of the GM events present in the test items is shown in Table 7. A significant proportion of laboratories did not quantify event 44406 (24 %, including 3 NRL/882) or VCO-1981 (34 %, including 5 NRL/882). All NRL/882 participants quantified at least one of both GM events.

The performance of those laboratories that had not reported a quantitative result for one or more of the events was not evaluated.

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

Quantitative Results Reported	Test Item 1 - 44406 Soybean		Test Item 2 - VCO-1981 Maize	
	NRL/882 and NRL/120[1]	Non-NRL	NRL/882 and NRL/120[1]	Non-NRL
Quantitative result	51	12	48	7
Measurement uncertainty	49	8	47	5
Coverage factor	44	7	40	5

[1] NRL/882 who have not reported a quantitative result for 44406 are L42, L54 (T1 was out of scope) and L56, and for VCO-1981, L22, L28, L48, L72 and L75.

A measurement uncertainty was reported for 92 % of all measurement results, with the coverage factor reported for 81 % of the results. These percentages have increased compared to those in previous CT rounds. It shows that most control laboratories understand the principle that analytical results should be reported with an uncertainty, when asked.

4.4.2 Assigned values

The assigned value (x_{pt}) for the mass fraction of event **44406 in T1** was based on the consensus value from the data from participants in this CT round, calculated using robust statistics[15,16]. This 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 some laboratories being experienced in GMO analysis, others not; these data were excluded to avoid introducing a bias in the calculation of the consensus value.

The results of proficiency tests for the analysis of GMOs are generally log-normally distributed (skewed)[17,18]; this was also the case for the 44406 results. To evaluate laboratory performance, the results reported by the NRLs (NRL/882 and NRL/120) were first $\log_{10}$-transformed, and the robust mean ($x_{pt\text{-}log}$) and corresponding robust standard deviation (s_R) 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[20] (section 7.7.3), as follows:

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

where: s_R = robust standard deviation of the results expressed in m/m % (on log scale);
N = number of data points 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[19].

For **VCO-1981 in T2**, the certified value (x_{CRM}) and its uncertainty [$u(x_{CRM})$] were taken from the certification report of ERM-BF438d[12] and were used to calculate the assigned value ($x_{pt\text{-}log}$) and its associated standard measurement uncertainty [$u(x_{pt\text{-}log})$]. The $\log_{10}$-transformation of the certified value (10 g/kg VCO-1981) gave the assigned value $x_{pt\text{-}log}$, while the corresponding standard measurement uncertainty was calculated following the standard equation for the expression of the measurement uncertainty on $\log_{10}$-transformed values, as follows:

$$u(x_{pt-log}) = 0.434 \frac{u(x_{CRM})}{x_{CRM}} \qquad (3)$$

where: x_{CRM} = the certified value of ERM-BF438d;
$u(x_{CRM})$ = the standard measurement uncertainty of the certified value, obtained by dividing the expanded measurement uncertainty by the coverage factor k = 2.

The assigned values and associated uncertainties for both GM events are reported in Table 8. The standard deviation for proficiency assessment ($\sigma_{pt\text{-}log}$) was set by the Advisory Board for CT to 0.15 (on the log scale) for both test items.

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

Variable	44406 Soybean	VCO-1981 Maize
Assigned value derived as	Robust mean of log_{10}-transformed data	Log_{10}-transformation of certified value
Number of data points (NRLs)	51	N.A.
Assigned Value ($x_{pt\text{-}log}$)	**-0.307**[1]	**0.000**[2]
Standard uncertainty [$u(x_{pt\text{-}log})$]	0.035	0.017
Standard deviation for proficiency assessment ($\sigma_{pt\text{-}log}$)	0.15	0.15

[1] The assigned value for 44406 corresponds to an approximate GM % in the raw domain of 0.51 m/m %, calculated by robust statistics on the raw data reported. Calculating the robust mean on the raw data gives, however, not exactly the same value as calculating the robust mean on the log_{10}-transformed data ($x_{pt\text{-}log}$) because the data distribution is different between both cases.
[2] The assigned value for VCO-1981 corresponds to a GM % in the raw domain of 1.00 m/m % because the $x_{pt\text{-}log}$ is the log_{10}-transformation of the certified value (10.0 g/kg).

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[20], 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 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.

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 scores was done according to ISO 17043:2010[5]:

|score| ≤ 2.0 satisfactory performance (green in Annex 4);
2.0 < |score| < 3.0 questionable performance (yellow in Annex 4);
|score| ≥ 3.0 unsatisfactory performance (red in Annex 4).

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 (after log transformation) 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 $-2.0 \leq z \leq 2.0$. These percentages may change when the true interlaboratory variability deviates from the standard deviation of 0.15, set based on experience from previous CT rounds and reasonable performance expectations. 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 not 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 CT scheme.

More detailed information about measurement uncertainty evaluation can be found in some international standards and other guidance documents[19,21,22,23,24].

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

Although the performance scores were calculated on the log-transformed data, the corresponding GM % on the raw domain, which are easier to understand, were as follows:

For 44406 soybean:

Assigned value on the raw domain	0.51 m/m %
$\|z\| \leq 2.0$ lower and upper limits	0.25 – 1.00 m/m %
$\|z\| < 3.0$ lower and upper limits	0.18 – 1.36 m/m %

For VCO-1981 maize:

Assigned value on the raw domain	1.00 m/m %
$\|z\| \leq 2.0$ lower and upper limits	0.50 – 2.03 m/m %
$\|z\| < 3.0$ lower and upper limits	0.37 – 2.77 m/m %

Table 9 summarises the performance results obtained in this CT round, 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
	44406 Soybean	VCO-1981 Maize
Number of laboratories with $\|z\| \leq 2.0$ (satisfactory)	53	55
Number of laboratories with $2.0 < \|z\| < 3.0$ (questionable)	5	0
Number of laboratories with $\|z\| \geq 3.0$ (unsatisfactory)	5	0

A total of 5 laboratories obtained an unsatisfactory performance, expressed as *z* score, and another 5 laboratories a questionable performance for event 44406. The unsatisfactory results were obtained by 1 NRL/882, 2 NRL/120 and 2 non-NRLs. For event VCO-1981, all laboratories received a satisfactory *z* score. In case of an unsatisfactory performance the laboratories will be requested to perform a root-cause analysis and to communicate the outcome to the EURL GMFF, who will then follow-up with the laboratory.

The performance of the laboratories for quantification of event 44406 soybean in soya milk powder was generally good, despite the difficulties for the extraction of good quality DNA from this matrix. During the production of soya milk the soybean DNA is highly degraded resulting in DNA fragments of relatively small molecular weight (MW); this is evidenced when analysing the extracted DNA by agarose gel electrophoresis (not shown). In contrast, the 44406 soybean spiked into this matrix was derived from a seed CRM and contained high MW DNA. This resulted in a sample to which a DNA extraction method was applied aiming to extract both the bulk of the low MW (non-GM) soybean DNA and the high MW 44406 soybean DNA with similar efficiencies. Although no experimental evidence is available, it would not be unexpected that some extraction methods and commercial kits for DNA purification would favour the recovery of higher MW DNA at the expense of the low MW fraction. Similarly, it may be envisaged that certain resins used in commercial purification columns do not efficiently bind the low MW DNA, and this may affect the balance between the (high MW) GM DNA and the (low MW) taxon-specific DNA.

The unsatisfactory performance for event 44406 corresponded to 4 overestimated results (> 1.95 m/m %) and one underestimated result (0.15 m/m %). Overestimation of the 44406 content may be due to a lower extraction efficiency or recovery of the taxon-specific DNA from the soy milk powder as a result of an inefficient precipitation of small DNA fragments or inefficient binding to the polymers used in commercial kits. Among the laboratories who have obtained an unsatisfactory performance expressed as *z* score, two had used a CTAB procedure, one the NucleoSpin Plant kit, and from the remaining two no information was available (questionnaire not submitted). Plotting the results from all participants against the DNA extraction method used showed that CTAB extraction (N = 21) resulted in 2 unsatisfactory results and 1 questionable result, NucleoSpin Food kit gave one questionable result while the other were satisfactory (N = 12), and Biotecon Foodproof gave 2 questionable results among N = 3 (and 1 additional questionable result was obtained by a laboratory reporting use of both NucleoSpin Plant and Biotecon Foodproof). All other extraction methods, when this information was reported in the questionnaire, gave satisfactory results. During a training workshop on DNA extraction that was organised by the EURL GMFF in June 2017, several participants had also communicated that quite deviating results were obtained on this soy milk matrix when using different DNA extraction methods. However, based on this evaluation and similar comparisons done for previous CT rounds, no firm conclusions could be taken with regard to the (un)suitability of certain DNA extraction methods for application to particular food or feed materials.

For VCO-1981, which was a seed-based matrix, and therefore it was easier to extract good quality DNA, the results reported were all very close to the assigned value of 1.0 m/m %. The $\sigma_{pt\text{-}log}$ used to calculate the *z* scores was in this case clearly larger than the true standard deviation of the reported results, which explains the absence of results that were not satisfactory.

4.4.4.2 ζ scores

Tables A4.1 and A4.2 also show the ζ scores obtained by the laboratories. A total of 35 laboratories were given a satisfactory performance when expressed as ζ score for 44406 (N = 63), 9 a questionable score and 19 an unsatisfactory score. For VCO-1981 (N = 55), 44 laboratories performed satisfactorily, 4 questionable and 7 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.

In Figures A4.1 and A4.2 (Annex 4) the reported measurement uncertainties can easily be compared between the participants; the figures also allow verification if the uncertainty bars overlap with the horizontal dashed line that corresponds to the satisfactory interval for the z scores. For 44406 soybean, it is clear that L36 has strongly overestimated its measurement uncertainty, and to a minor extent this also applies to L37, although they both obtained a satisfactory ζ score because their reported values were close to the assigned value. The same can be said for the VCO-1981 result reported by L86. Both L32 and L60 also reported quite large measurement uncertainties for 44406 soybean (and obtained unsatisfactory z and ζ scores), but this cannot be seen in the graph because their reported result (X_i) was also large (and this is the denominator in formula (6)). From the same figures, it can easily be seen that several laboratories have reported a too low measurement uncertainty (or no uncertainty at all) and therefore received an unsatisfactory ζ score; e.g. if L49 and L81 would have reported a realistic measurement uncertainty, and if L8, L31 and L71 would have reported an uncertainty value at all, they would have obtained a satisfactory ζ score for 44406 (Figure A4.1). Similarly, for VCO-1981 (Figure A4.2), L10, L31 and L71 have not reported the measurement uncertainty, and the results of L49, L62 and L66 would have been satisfactory if they had reported a more realistic measurement uncertainty.

As a general guideline, standard measurement uncertainties for both GM events below the measurement uncertainty of the assigned value (i.e. < 0.04 m/m % on the raw scale) are probably underestimated, while values above 1.5 times the robust standard deviation of the (NRL) results, i.e. above 0.35 m/m % for 44406 and above 0.25 m/m % for VCO-1981, may be overestimated[20].

5 Conclusions

Participants in this CT round were required to analyse two test items varying in composition and complexity. The analytical tasks resembled the routine operational analysis tasks of an official control laboratory analysing a food or feed material for the presence of 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. The majority of the participants performed satisfactorily for all tasks in this CT round, *i.e.* the detection and quantification of the soybean event 44406 in T1, a soya milk powder, and VCO-1981 maize in T2, a maize flour. All participants who tested for the events were able to identify the correct events in both test items. Regarding quantification, five laboratories, including one NRL/882, obtained an unsatisfactory *z* score for 44406 soybean measurements in a more difficult food matrix. All laboratories demonstrated a satisfactory performance for quantification of event VCO-1981 in the T2 matrix.

While all laboratories demonstrated a satisfactory performance for analysis of a relatively simple matrix (T2), the challenging matrix of T1 affected the performance of some of the laboratories for quantification of the event in T1. The soy milk powder of test item T1 was also somewhat artificial in terms of the combination of low MW soybean DNA and high MW DNA from the spiked 44406 soybean.

One third of the participants had not provided a quantitative result, including 6 and 5 NRL/882 for events 44406 and VCO-1981, respectively.

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

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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
	ANSES	LSV	Angers cedex 01	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 [2]		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 España 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
	Landesuntersuchungsamt	Institut f. Lebensmittelchemie	Trier	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
	Hessisches Landeslabor		Kassel	GERMANY
	Landeslabor Schleswig-Holstein		Neumünster	GERMANY

Body	Organisation	Department	City	Country
NRL/120 cont.	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GB 6, Fachbereich 63	Nossen	GERMANY
	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
	Executive Environment Agency	LBM and GMO	Sofia	BULGARIA
	Laboratory of SGS Bulgaria Ltd		Varna	BULGARIA
	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
	Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe		Münster	GERMANY
	Biomi Ltd.		Godollo	HUNGARY
	IZSLER		Brescia	ITALY
	Istituto Sperimentale Del Piemonte, Liguria e Valle D'Aosta	S.C. Biotechnologie	Torino	ITALY
	Istituto Zooprofilattico Sperimentale Del Mezzogiorno	Food Control	Portici	ITALY
	Department of Chemistry Malaysia	Biotechnology Section	Petaling Jaya	MALAYSIA
	SENASICA-CNRDOGM	Detección de OGM	Tecámac	MEXICO
	Wojewodzki Inspektorat Weterynarii	Zaklad Higieny Weterynaryjnej	Opole	POLAND
	Laboratorul Central pentru Calitatea Semintelor si a Materialului Saditor Bucuresti	LEDOMG	Bucuresti	ROMANIA
	SP Laboratorija a.d.	Genetical dpt.	Becej	SERBIA
	Agri-Food & Veterinary Authority of Singapore	Veterinary Public Health Labor	Singapore	SINGAPORE
	University of the Free State	GMO Testing Facility G2	Bloemfontein	SOUTH AFRICA
	Federal Food Safety and Veterinary Office FSVO	Risk Assessment Division	Bern	SWITZERLAND
	Agroscope	Feed Analytics	Posieux	SWITZERLAND
	DNA Technology Laboratory		Nakorn Pathom	THAILAND
	National Gene Bank of Tunisia	GMO testing Laboratory	Tunis	TUNISIA
	National Food Reference Laboratory	Biotechnology and GMO Unit	Ankara	TURKEY
	Ankara Food Control Laboratory	Molecular Biology	Ankara	TURKEY
	Ukrmetrteststandart	Molecular Biology	Kiev	UKRAINE
	Ukrainian Laboratory of Quality and Safety of Agricultural Products (ULQSAP)		Chabany village	UKRAINE
	Worcestershire Scientific Services		Worcester	UNITED KINGDOM
	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 carry out official controls for GMO 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.

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

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List of tables

(Table numbers preceded by an "A" refer to tables in an Annex)

List of figures

(Figure numbers preceded by an "A" refer to figures in an Annex)

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[20] 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[25] and σ_{pt} is the standard deviation for comparative testing. The value of σ_{pt}, the target standard deviation for comparative testing, was defined by the Members of the Advisory Board on the basis of the experience acquired in previous CT rounds, and set to 0.15 on the log domain[26]. On a raw data scale, this σ_{pt} value corresponds to approximately 0.15 m/m % (30 % of x_{pt}).

If the criterion according to A1.1 is met (i.e. $s_s \leq 0.045$), 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 0.024 m/m %. The criterion described in formula (A1.1) was fulfilled (0.024 < 0.045), indicating that T1 was homogeneous.

A1.2 Stability of test items

For T1, an isochronous short-term stability study[27] 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 44406 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 soybean event 44406. Even at 60 °C, no significant trend was measured.

The test items were shipped at ambient temperature.

The stability of T1 during the period covered by the CT 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-BF438. 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 75 laboratories were exported from the EUSurvey "Questionnaire on CT 01/17 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 present 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		30	40%
NRL/120		17	22.67%
Non-NRL		28	37.33%
No Answer		0	0%

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

		Answers	Ratio
T1 was not analysed: go to Q1		6	8%
T1 was analysed: go to Q2		69	92%
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		2	2.67%
b) The methods are not validated in our laboratory		2	2.67%
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)		1	1.33%
e) We tried but our analysis failed		0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)		1	1.33%
g) Other reason		0	0%
No Answer		69	92%

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

		Answers	Ratio
CTAB		30	40%
NucleoSpin Food		12	16%
NucleoSpin Plant		2	2.67%
GeneSpin		6	8%
Promega Wizard		3	4%
DNeasy Plant		0	0%
DNeasy Mericon Food		2	2.67%
Biotecon Foodproof		5	6.67%
SDS		2	2.67%
Fast ID Genomic DNA		2	2.67%
Maxwell 16 Plant DNA		0	0%
Maxwell 16 Food, Feed, Seed		5	6.67%
Generon Ion Force		2	2.67%
Other		3	4%
No Answer		6	8%

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

		Answers	Ratio
No additional clean-up		45	60%
Additional ethanol precipitation		10	13.33%
Eurofins DNAExtractor cleaning column		4	5.33%
Promega Wizard DNA clean-up resin		5	6.67%
Qiagen QIAQuick		3	4%
Qiagen Genomic-Tip 20/G		0	0%
Other method (no need to specify)		4	5.33%
No Answer		6	8%

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

		Answers	Ratio
1		0	0%
2		44	58.67%
3		8	10.67%
4		10	13.33%
5		1	1.33%
6		3	4%
>6		3	4%
No Answer		6	8%

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

		Answers	Ratio
None (no inhibition was suspected based on experience)		3	4%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		36	48%
We verify that the amplification curves look normal		18	24%
We run two dilutions and verify if the delta Cq or GM% are as expected		31	41.33%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		10	13.33%
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		12	16%
We add an internal positive control to the reactions and check the Cq		11	14.67%
Other		1	1.33%
No Answer		6	8%

T1: 6. Select the option applicable to your analysis for 44406 soybean in T1?

		Answers	Ratio
Not tested		8	10.67%
Detected but not quantified		6	8%
Detected and quantified: please fill in Q6a-6h		55	73.33%
Found absent		0	0%
No Answer		6	8%

T1: 7. Select the option applicable to your analysis for CV127 soybean in T1?

		Answers	Ratio
Not tested		4	5.33%
Detected but not quantified		1	1.33%
Detected and quantified: please fill in Q7a-7h		0	0%
Found absent		64	85.33%
No Answer		6	8%

T1: 8. Select the option applicable to your analysis for MON87708 soybean in T1?

		Answers	Ratio
Not tested		8	10.67%
Detected but not quantified		1	1.33%
Detected and quantified: please fill in Q8a-8h		0	0%
Found absent		60	80%
No Answer		6	8%

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		53	70.67%
b) The event-specific detection method is not validated in our laboratory		8	10.67%
c) Reference material, primers, probes, or other reagents were not available (in time)		11	14.67%
d) The result obtained was below the LOD/LOQ		1	1.33%
e) Practical constraints (instrument broken, no personnel, etc.)		0	0%
f) Other reason		2	2.67%
No Answer		6	8%

T1: 6.a. Soybean 44406: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		47	62.67%
Delta Cq method (one calibration curve)		7	9.33%
Digital PCR (no calibration curve)		2	2.67%
No Answer		20	26.67%

T1: 6.b. Select the calibrant used for the 44406 standard curve.

		Answers	Ratio
CRM from IRMM, certified in GM mass fraction (g/kg)		52	69.33%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction (g/kg or m/m %)		1	1.33%
Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		0	0%
No calibrant used, digital PCR done		2	2.67%
No Answer		20	26.67%

T1: 6.c. Select the endogenous target(s) used for relative quantification of 44406 soybean in T1.

		Answers	Ratio
Soybean lec 74 bp (40-3-2, MON89788, MON87701, 44406, 356043, 305423, etc.)		49	65.33%
Soybean lec 81 bp (Pauli et al., 2001)		2	2.67%
Soybean lec 102 bp (A5547, FG72)		1	1.33%
Soybean lec 105 bp (A2704)		1	1.33%
Soybean lec 118 bp (Shindo et al., 2002)		1	1.33%
Other, please specify below		1	1.33%
No Answer		20	26.67%

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

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 44406. 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.		37	49.33%
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).		16	21.33%
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.		1	1.33%
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). No conversion factor was applied onto the final GM %.		1	1.33%
No Answer		20	26.67%

Conversion factor used to turn results into m/m %, if applicable, and/or clarification on preparation of standards.
L39: 0.95 (digital PCR)

T1: 6.e. What was the amount of sample DNA (ng) used per PCR for 44406. 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		6	8%
250 ng		3	4%
200 ng		16	21.33%
150 ng		6	8%
100 ng		22	29.33%
50 ng		13	17.33%
25 ng		6	8%
15 ng		1	1.33%
<10 ng		0	0%
No Answer		20	26.67%

T1: 6.f. What was the LOQ (in m/m %) for the 44406 quantification (if applicable, otherwise leave blank)?

	Answers
0.02	2
0.03	4
0.04	2
0.05	3
0.06	1
0.07	1
0.08	1
0.09	7
0.1	31
0.12	1
0.16	1

T1: 6.g. How was the LOQ for 44406 determined (if applicable)?

		Answers	Ratio
Determined from the qPCR analysis for the current sample		19	25.33%
Determined during the in-house validation of the method		17	22.67%
Taken from the EURL GMFF validation report		23	30.67%
By another approach, please explain below		2	2.67%
No Answer		20	26.67%

Explanation on alternative LOQ determination:
The method is not in-house validated quantitatively, only qualitatively.
Determined from the digital PCR Analysis for the current sample

T1: 6.h. How did you estimate the measurement uncertainty on the result reported for 44406 soybean?

		Answers	Ratio
Uncertainty budget (ISO GUM)		2	2.67%
Uncertainty of the method (in-house validation)		12	16%
Known uncertainty of the standard method		3	4%
Measurement of replicates (precision)		29	38.67%
From interlaboratory comparison data		3	4%
Estimation based on judgement		2	2.67%
In another way, please specify below		7	9.33%
No Answer		20	26.67%

Explanation on alternative determination of measurement uncertainty:
Combined uncertainty (CRM+measurement) following Application note 1 (Linsinger, 2005, JRC Geel)
The u was obtain through the estimation of the sd taking into account the repeatability and intermediate precision associated with the test sample. Then U was calculated taking into account u of used calibrant. An appropriate coverage factor was used based on the degrees of freedom (95% CI).
Measurement uncertainty was not calculated. If calculated, it would be calculated from the repeatability.
95% confidence Interval of the results for the current sample
Calculation of MU from collaborative trial data, according to: JRC Scientific and Technical Reports: Guidance Document on Measurement Uncertainty for GMO Testing Laboratories (EUR 22756 EN/2 - 2009)
According course material of JRC: GMO Quantification: Proper calibration and Estimation of Measurement Uncertainty (2013)
Internal quality control data : within-laboratory reproducibility + absence of bias
Uncertainty = coverage factor (P=95% and f=n-1) * Standard Deviation / Square-root (Number of measurement); coverage factor (P=95%, f=5) = 2,57
U=S/a √1/p+1/n+(c0-c)2/Sxx

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

		Answers	Ratio
T2 was not analysed: go to Q1		0	0%
T2 was analysed: go to Q2		75	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		75	100%

Additional comments and suggestions
OUR LABORATORY SUBMITS ONLY QUALITATIVE RESULTS
The method is not in-house validated quantitatively, only qualitatively.
GTS 40-3-2 was detected in traces at the LOD
With the NucleoSpin Food Kit we received approx. 0.4 m/m % of DAS44406-6. The quantified reference gene target is equal for samples prepared with both extraction kits. Because of this fact we give the result of the samples with the higher amount of % GMO.

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

		Answers	Ratio
CTAB		37	49.33%
NucleoSpin Food		10	13.33%
NucleoSpin Plant		4	5.33%
GeneSpin		5	6.67%
Promega Wizard		5	6.67%
DNeasy Plant		1	1.33%
DNeasy Mericon Food		1	1.33%
Biotecon Foodproof		4	5.33%
SDS		2	2.67%
Fast ID Genomic DNA		2	2.67%
Maxwell 16 Plant DNA		0	0%
Maxwell 16 Food, Feed, Seed		4	5.33%
Generon Ion Force		2	2.67%
Other		4	5.33%
No Answer		0	0%

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

		Answers	Ratio
No additional clean-up		47	62.67%
Additional ethanol precipitation		11	14.67%
Eurofins DNAExtractor cleaning column		3	4%
Promega Wizard DNA clean-up resin		7	9.33%
Qiagen QIAQuick		4	5.33%
Qiagen Genomic-Tip 20/G		0	0%
Other method (no need to specify)		5	6.67%
No Answer		0	0%

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

		Answers	Ratio
1		0	0%
2		49	65.33%
3		7	9.33%
4		12	16%
5		1	1.33%
6		4	5.33%

>6		2	2.67%
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)		5	6.67%
We run two dilutions and verify if the delta Cq or GM% are as expected		33	44%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		9	12%
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		10	13.33%
We add an internal positive control to the reactions and check the Cq		11	14.67%
We verify that the amplification curves look normal		17	22.67%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		33	44%
Other		1	1.33%
No Answer		0	0%

T2: 6. Select the option applicable to your analysis for MON810 maize in T1?

		Answers	Ratio
Not tested		0	0%
Detected but not quantified		2	2.67%
Detected and quantified: please fill in Q6a-6h		0	0%
Found absent		73	97.33%
No Answer		0	0%

T2: 7. Select the option applicable to your analysis for NK603 maize in T1?

		Answers	Ratio
Not tested		0	0%
Detected but not quantified		2	2.67%
Detected and quantified: please fill in Q7a-7h		0	0%
Found absent		73	97.33%
No Answer		0	0%

T2: 8. Select the option applicable to your analysis for VCO-1981 maize in T1?

		Answers	Ratio
Not tested		19	25.33%
Detected but not quantified		6	8%
Detected and quantified: please fill in Q8a-8h		50	66.67%
Found absent		0	0%
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		50	66.67%
b) The event-specific detection method is not validated in our laboratory		13	17.33%
c) Reference material, primers, probes, or other reagents were not available (in time)		15	20%
d) The result obtained was below the LOD/LOQ		1	1.33%
e) Practical constraints (instrument broken, no personnel, etc.)		0	0%
f) Other reason		3	4%
No Answer		0	0%

T2: 8.a. VCO-1981 maize: Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		44	58.67%
Delta Cq method (one calibration curve)		7	9.33%
Digital PCR (no calibration curve)		0	0%
No Answer		25	33.33%

T2: 8.b. Select the calibrant used for the VCO-1981 standard curve.

		Answers	Ratio
CRM from IRMM, certified in GM mass fraction (g/kg)		49	65.33%
Non-certified RM (e.g. lab QC material), expressed in GM mass fraction		1	1.33%

Non-certified RM (e.g. lab QC material), expressed in GM DNA copy number ratio (e.g. determined by digital PCR)		0	0%
No calibrant used, digital PCR done		0	0%
No Answer		25	33.33%

T2: 8.c. Select the endogenous target(s) used for relative quantification of VCO-1981 maize.

		Answers	Ratio
Hmg		31	41.33%
Adh1-70 bp		1	1.33%
Adh1-134 to 136 bp		3	4%
zSSIIb		0	0%
Zein		0	0%
ivr		0	0%
Aldolase		15	20%
Other, please specify below		0	0%
No Answer		25	33.33%

T2: 8.d. Clarify the unit of measurement used and any conversion between units if applicable. Carefully read the choices below and select the one used in the measurements that resulted in a final result in GM m/m % for VCO-1981. 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		37	49.33%
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)		11	14.67%
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.		4	5.33%
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.		1	1.33%
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 %.		0	0%
No Answer		25	33.33%

T2: 8.e. What was the amount of DNA (ng) used per PCR for VCO-1981? 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		6	8%
250 ng		3	4%
200 ng		18	24%
150 ng		9	12%
100 ng		15	20%
50 ng		14	18.67%
25 ng		5	6.67%
15 ng		1	1.33%
<10 ng		0	0%
No Answer		25	33.33%

T2: 8.f. What was the LOQ (in m/m %) for the VCO-1981 quantification (if applicable, otherwise leave blank)?

	Answers
0.02	2
0.03	1
0.05	4
0.06	2
0.07	3
0.09	7
0.1	24
0.12	1
0.2	1
0.4	

T2: 8.g. How was the LOQ for VCO-1981 determined (if applicable)?

		Answers	Ratio
Determined from the qPCR analysis for the current sample		20	26.67%
Determined during the in-house validation of the method		14	18.67%
Taken from the EURL GMFF validation report		21	28%
By another approach, please explain below		1	1.33%
No Answer		25	33.33%

Explanation on alternative LOQ determination:
The method is not inhouse-validated.

T2: 8.h. How did you estimate the measurement uncertainty on the result reported for VCO-1981 maize?

		Answers	Ratio
Uncertainty budget (ISO GUM)		2	2.67%
Uncertainty of the method (in-house validation)		10	13.33%
Known uncertainty of the standard method		3	4%
Measurement of replicates (precision)		26	34.67%
From interlaboratory comparison data		3	4%
Estimation based on judgement		3	4%
In another way, please specify below		6	8%
No Answer		25	33.33%

Explanation on alternative determination of measurement uncertainty:
Combined uncertainty (CRM+measurement) following Application note 1 (Linsinger, 2005, JRC Geel)
The u was obtain through the estimation of the sd taking into account the repeatability and intermediate precision associated with the test sample. Then U was calculated taking into account u of used calibrant. An appropriate coverage factor was used based on the degrees of freedom (95% CI).
95% confidence Interval of the results for the current sample
30% of the calculated value
Internal quality control data : within-laboratory reproducibility + absence of bias
Uncertainty = coverage factor (P=95% and f=n-1) * Standard Deviation / Square-root (Number of measurement); coverage factor (P=95%, f=5) = 2,57

Additional comments and suggestions
For T1 and T2 items we analysed p35S, tNOS, pFMV by screening. T1 item includes p35S, tNOS and pFMV but T2 item doesn't include any parameters. Also T1 item includes 40-3-2 soybean and MON89788 soybean but their amount of <LOQ.
We need a ct-round with MON87701 in feed, with quantification of MON87701!!
OUR LABORATORY SUBMITS ONLY QUALITATIVE RESULTS
The method is not in-house validated.
We did not found event MON810 and NK603 in sample T2. For the event VCO-1981, we don't have reference material, primers and probes.
Measurement of uncertainty determined from standard deviation of 8 independent measurements, coverage factor 2
According course material of JRC: GMO Quantification: Proper calibration and Estimation of Measurement Uncertainty (2013)
T2: 8.d - We applied the conversion factor 0.5, to covert from cp/cp to m/m %, as specified in "Technical guidance document from the EURL GMFF - Reg 619/2011.
The method for quantification of VCO-01891 is being verified in the laboratory and not yet under the scope of accreditation
DNA isolation T2 with CTAB extraction followed by Qiagen DNeasy Plant kit
The uncertainty value of 0.9 was estimated from the repeatability standard deviation of the test item measurements.

Annex 3: Qualitative performance of the participants

Table A3.1. Performance of the participants for the qualitative identification of GM events in comparative test EURL-GMFF-CT-01/17.

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

Lab	44406 detected	VCO-1981 detected	Lab	44406 detected	VCO-1981 detected
L01	yes	yes	**L45**	yes	yes
L02	yes	yes	**L46**	yes*	?**
L03	yes	yes	**L47**	T1 was not analysed	Not tested
L04	Not tested	Not tested	**L48**	yes	Not tested
L05	yes	yes	**L49**	yes*	yes*
L06	yes	Not tested	**L50**	T1 was not analysed	yes
L07	yes	yes	**L51**	yes	yes
L08	yes	Not tested	**L52**	T1 was not analysed	Not tested
L09	Not tested	Not tested	**L53**	yes	yes
L10	yes	yes	**L54**	T1 was not analysed	yes
L11	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	Not tested	Not tested	**L63**	yes	yes
L20	yes	yes	**L66**	yes	yes
L22	yes	yes	**L67**	yes	yes
L24	yes*	yes*	**L68**	yes	yes
L25	yes	yes	**L69**	yes	yes
L26	yes	Not tested	**L70**	Not tested	Not tested
L27	yes	yes	**L71**	yes	yes
L28	yes	yes	**L72**	yes	Not tested
L29	yes	yes	**L73**	T1 was not analysed	Not tested
L30	yes	yes	**L74**	Not tested	Not tested
L31	yes	yes	**L75**	yes	yes
L32	yes*	?	**L76**	Not tested	Not tested
L33	yes	yes	**L78**	yes	yes
L34	yes	yes	**L79**	yes	yes
L35	yes	yes	**L80**	Not tested	Not tested
L36	yes	Not tested	**L81**	yes	yes
L37	yes	Not tested	**L82**	yes	yes
L38	yes	yes	**L83**	yes	yes
L39	yes	yes	**L84**	yes	yes
L40	Not tested	Not tested	**L85**	T1 was not analysed	Not tested
L41	yes	yes	**L86**	yes	yes
L42	yes	yes	**L87**	yes	yes
L43	yes	yes	**L88**	yes	yes
L44	yes*	yes*			

* Although the questionnaire was not returned by the participant, the presence of the event was inferred from the quantitative result reported.
** A question mark indicates that no information was available on the presence, absence or absence of testing of the event.

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 44406 soybean and VCO-1981 maize, respectively. For consistency, the reported results are shown with two decimals; if not reported, the cell is left blank. The performance 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 for 44406 soybean in soya milk powder (T1)[1].

	44406
$X_{pt\text{-}log}$	-0.307
$u(x_{pt\text{-}log})$	0.035
$\sigma_{pt\text{-}log}$	0.15

Category	Lab	x_i	U	k	$u(x_i)$	$\log(x_i)$	z score	ζ score
NRL/120	L01	0.44	0.15	2.00	0.08	-0.3565	-0.3	-0.6
Non-NRL	L02	0.49	0.23	2.00	0.12	-0.3098	0.0	0.0
NRL/882	L03	0.93	0.19	2.00	0.10	-0.0315	1.8	**4.9**
NRL/882	L05	0.55	0.15	2.09	0.07	-0.2596	0.3	0.7
NRL/882	L07	0.36	0.17	2.09	0.08	-0.4437	-0.9	-1.3
Non-NRL	L08	0.32				-0.4949	-1.2	**-5.3**
Non-NRL	L10	0.50				-0.3010	0.0	0.2
NRL/120	L11	1.95	0.03	2.00	0.02	0.2900	**4.0**	**16.9**
NRL/882	L12	0.41	0.09	*	0.05	-0.3872	-0.5	-1.2
NRL/120	L13	0.15	0.02	2.00	0.01	-0.8239	**-3.4**	**-11.3**
NRL/120	L14	0.41	0.03	2.00	0.02	-0.3872	-0.5	-2.1
NRL/882	L15	0.60	0.34	2.00	0.17	-0.2218	0.6	0.7
NRL/882	L16	0.42	0.16	2.00	0.08	-0.3768	-0.5	-0.8
NRL/882	L17	0.32	0.08	2.00	0.04	-0.4949	-1.2	-2.9
NRL/882	L18	0.64	0.20	2.00	0.10	-0.1938	0.8	1.5
NRL/120	L20	1.02	0.29	2.23	0.13	0.0086	2.1	**4.8**
NRL/882	L22	0.50	0.15	2.00	0.08	-0.3010	0.0	0.1
NRL/882	L24	0.21	0.08	2.00	0.04	-0.6778	-2.5	**-4.1**
NRL/882	L25	0.60	0.18	*	0.10	-0.2218	0.6	1.0
Non-NRL	L26	0.50				-0.3010	0.0	0.2
NRL/882	L27	0.29	0.07	2.00	0.04	-0.5376	-1.5	**-3.6**
NRL-882	L28	1.10				0.0414	2.3	**9.9**
NRL/120	L29	0.38	0.08	2.00	0.04	-0.4202	-0.8	-2.0
NRL/120	L30	0.45	0.14	2.00	0.07	-0.3468	-0.3	-0.5
NRL/882	L31	0.41				-0.3872	-0.5	-2.3
Non-NRL	L32	4.85	1.21	*	0.70	0.6857	**6.6**	**13.8**
NRL/120	L33	0.33	0.14	2.31	0.06	-0.4815	-1.2	-2.0
NRL/882	L34	0.96	0.08	2.00	0.04	-0.0177	1.9	**7.3**
NRL/882	L35	0.93	0.45	2.00	0.23	-0.0315	1.8	2.5
Non-NRL	L36	0.71	2.06	2.00	1.03	-0.1487	1.1	0.3
Non-NRL	L37	0.74	0.66	2.00	0.33	-0.1337	1.2	0.9
Non-NRL	L38	0.66	0.11	2.00	0.06	-0.1805	0.8	2.5
NRL/120	L39	0.43	0.04	2.00	0.02	-0.3665	-0.4	-1.5
NRL/882	L41	0.36	0.18	2.00	0.09	-0.4437	-0.9	-1.2

Category	Lab	x_i	U	k	$u(x_i)$	$\log(x_i)$	z score	ζ score
NRL/882	L43	0.98	0.16	2.00	0.08	-0.0088	2.0	**6.0**
NRL/882	L44	0.81	0.24	2.00	0.12	-0.0915	1.4	2.9
NRL/120	L46	0.30	0.10	2.20	0.05	-0.5229	-1.4	-2.9
NRL/882	L48	0.35	0.14	2.00	0.07	-0.4559	-1.0	-1.6
NRL/120	L49	0.65	0.04	3.00	0.01	-0.1871	0.8	**3.3**
NRL/882	L51	0.46	0.07	2.00	0.04	-0.3372	-0.2	-0.6
NRL/120	L53	0.30	0.10	2.00	0.05	-0.5229	-1.4	-2.7
NRL/120	L55	0.43	0.10	2.00	0.05	-0.3665	-0.4	-1.0
NRL/120	L57	0.59	0.20	2.00	0.10	-0.2291	0.5	1.0
Non-NRL	L58	0.53	0.10	2.00	0.05	-0.2757	0.2	0.6
Non-NRL	L59	0.54	0.11	2.00	0.06	-0.2676	0.3	0.7
NRL/882	L60	2.00	0.87	2.23	0.39	0.3010	**4.1**	**6.6**
NRL/882	L61	0.43	0.17	2.00	0.09	-0.3665	-0.4	-0.6
NRL/120	L62	0.52	0.05	*	0.03	-0.2840	0.2	0.5
Non-NRL	L63	2.06	0.31	2.00	0.16	0.3139	**4.1**	**12.9**
NRL/120	L66	0.52	0.04	*	0.02	-0.2840	0.2	0.6
NRL/120	L67	0.47	0.14	2.00	0.07	-0.3279	-0.1	-0.3
NRL/882	L68	0.46	0.09	2.00	0.05	-0.3372	-0.2	-0.5
NRL/120	L69	0.80	0.18	2.00	0.09	-0.0969	1.4	**3.5**
Non-NRL	L71	0.33				-0.4815	-1.2	**-4.9**
NRL/882	L72	0.35	0.08	2.00	0.04	-0.4559	-1.0	-2.4
NRL/882	L75	1.02	0.18	2.00	0.09	0.0086	2.1	**6.1**
NRL/882	L78	0.42	0.13	2.00	0.07	-0.3768	-0.5	-0.9
NRL/882	L81	0.31	0.06	2.00	0.03	-0.5086	-1.3	**-3.7**
NRL/120	L82	0.18	0.07	2.00	0.03	-0.7447	-2.9	**-5.0**
NRL/882	L83	0.47	0.13	2.00	0.07	-0.3279	-0.1	-0.3
NRL/120	L84	0.49	0.05	*	0.03	-0.3098	0.0	-0.1
NRL/882	L86	0.45	0.05	2.00	0.03	-0.3468	-0.3	-0.9
NRL/120	L87	0.43	0.07	2.00	0.04	-0.3665	-0.4	-1.2

[1] NRL/882 who have not reported a quantitative result for 44406 are L42, L54 (T1 was out of scope) and L56.
* The k factor was not reported by the laboratory; a value of 1.73 was assigned for calculation of the ζ score.

Table A4.2. Quantitative results (in m/m %) and performance scores of participants for VCO-1981 maize in maize flour (T2).

	$X_{pt\text{-}log}$	**0.000**
	$u(x_{pt\text{-}log})$	**0.017**
	$\sigma_{pt\text{-}log}$	**0.15**

Category	Lab	x_i	U	k	$u(x_i)$	$\log(x_i)$	z score	ζ score
NRL/120	L01	1.35	0.46	2.00	0.23	0.1303	0.9	1.7
Non-NRL	L02	1.07	0.45	2.00	0.23	0.0294	0.2	0.3
NRL/882	L03	1.00	0.13	2.00	0.07	0.0000	0.0	0.0
NRL/882	L05	0.97	0.29	2.05	0.14	-0.0132	-0.1	-0.2
NRL/882	L07	0.96	0.34	2.12	0.16	-0.0177	-0.1	-0.2
Non-NRL	L10	0.86				-0.0655	-0.4	**-3.8**
NRL/882	L12	1.30	0.27	*	0.16	0.1139	0.8	2.1
NRL/120	L13	1.09	0.10	2.00	0.05	0.0374	0.2	1.4
NRL/120	L14	0.99	0.10	2.00	0.05	-0.0044	0.0	-0.2
NRL/882	L15	1.55	0.33	2.00	0.17	0.1903	1.3	**3.9**
NRL/882	L16	1.42	0.54	2.00	0.27	0.1523	1.0	1.8
NRL/882	L17	1.01	0.19	2.00	0.10	0.0043	0.0	0.1
NRL/882	L18	1.01	0.30	2.00	0.15	0.0043	0.0	0.1
NRL/120	L20	1.17	0.29	2.23	0.13	0.0682	0.5	1.3
NRL/882	L24	1.29	0.44	2.00	0.22	0.1106	0.7	1.5
NRL/882	L25	1.27	0.38	*	0.22	0.1038	0.7	1.3
NRL/882	L27	0.99	0.25	2.00	0.13	-0.0044	0.0	-0.1
NRL/120	L29	1.07	0.10	2.00	0.05	0.0294	0.2	1.1
NRL/120	L30	1.30	0.39	2.00	0.20	0.1139	0.8	1.7
NRL/882	L31	1.59				0.2014	1.3	**11.6**
NRL/120	L33	1.07	0.35	2.57	0.14	0.0294	0.2	0.5
NRL/882	L34	0.99	0.15	2.00	0.08	-0.0044	0.0	-0.1
NRL/882	L35	1.08	0.28	2.00	0.14	0.0334	0.2	0.6
Non-NRL	L38	1.09	0.38	2.00	0.19	0.0374	0.2	0.5
NRL/120	L39	1.09	0.10	2.00	0.05	0.0374	0.2	1.4
NRL/882	L41	1.00	0.27	2.00	0.14	0.0000	0.0	0.0
NRL/882	L42	1.02	0.26	*	0.15	0.0086	0.1	0.1
NRL/882	L43	0.96	0.30	2.00	0.15	-0.0177	-0.1	-0.3
NRL/882	L44	0.84	0.24	2.00	0.12	-0.0757	-0.5	-1.2
NRL/120	L49	1.26	0.08	3.00	0.03	0.1004	0.7	**5.1**
NRL/120	L50	1.00	0.20	2.00	0.10	0.0000	0.0	0.0
NRL/882	L51	1.00	0.13	2.00	0.07	0.0000	0.0	0.0
NRL/120	L53	0.71	0.28	2.00	0.14	-0.1487	-1.0	-1.7
NRL/882	L54	0.90	0.30	*	0.17	-0.0458	-0.3	-0.5
NRL/120	L55	1.02	0.27	2.00	0.14	0.0086	0.1	0.1
NRL/882	L56	1.09	0.23	2.00	0.12	0.0374	0.2	0.8
NRL/120	L57	1.10	0.40	2.00	0.20	0.0414	0.3	0.5
Non-NRL	L58	0.92	0.31	2.00	0.16	-0.0362	-0.2	-0.5
Non-NRL	L59	0.57	0.10	2.00	0.05	-0.2441	-1.6	**-5.8**
NRL/882	L60	0.81	0.29	2.23	0.13	-0.0915	-0.6	-1.3
NRL/882	L61	0.90	0.36	2.00	0.18	-0.0458	-0.3	-0.5
NRL/120	L62	1.09	0.01	*	0.01	0.0374	0.2	2.1
Non-NRL	L63	2.03	0.30	2.00	0.15	0.3075	2.0	**8.4**
NRL/120	L66	1.28	0.14	*	0.08	0.1072	0.7	**3.3**

Category	Lab	x_i	U	k	$u(x_i)$	$\log(x_i)$	z score	ζ score
NRL/120	L67	1.17	0.13	2.00	0.07	0.0682	0.5	2.3
NRL/882	L68	0.95	0.27	2.00	0.14	-0.0223	-0.1	-0.3
NRL/120	L69	0.92	0.25	2.00	0.13	-0.0362	-0.2	-0.6
Non-NRL	L71	1.10				0.0414	0.3	2.4
NRL/882	L78	0.91	0.27	2.00	0.14	-0.0410	-0.3	-0.6
NRL/882	L81	1.23	0.43	2.00	0.22	0.0899	0.6	1.2
NRL/120	L82	1.11	0.52	2.00	0.26	0.0453	0.3	0.4
NRL/882	L83	0.89	0.25	2.00	0.13	-0.0506	-0.3	-0.8
NRL/120	L84	0.93	0.09	*	0.05	-0.0315	-0.2	-1.1
NRL/882	L86	0.90	0.90	2.00	0.45	-0.0458	-0.3	-0.2
NRL/120	L87	0.94	0.19	2.00	0.10	-0.0269	-0.2	-0.6

[1] NRL/882 who have not reported a quantitative result for VCO-1981, L22, L28, L48, L72 and L75.
* The k factor was not reported by the laboratory; a value of 1.73 was assigned for calculation of the ζ score.

Figure A4.1. Laboratory results for soybean event 44406 in test item 1.

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

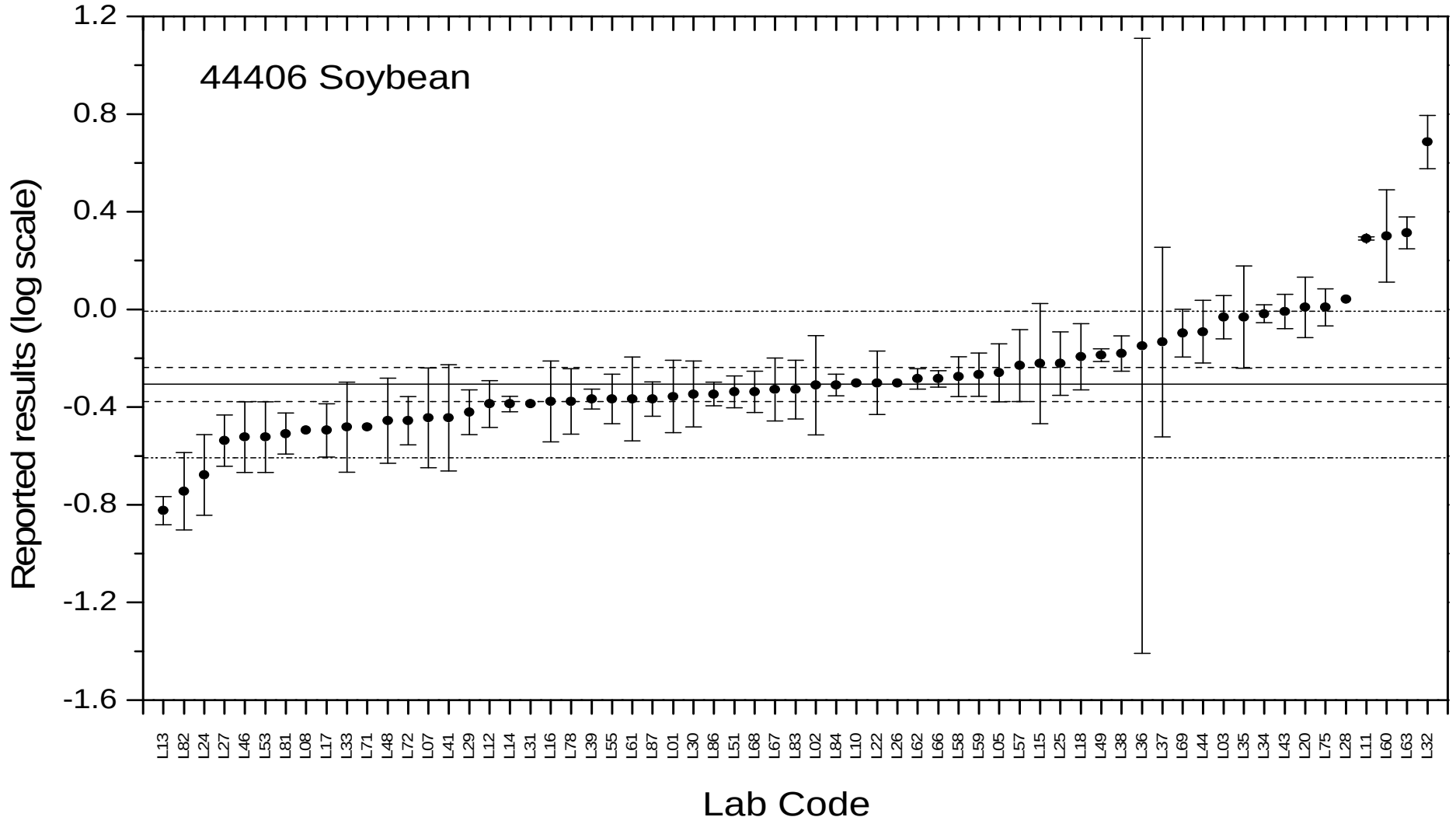
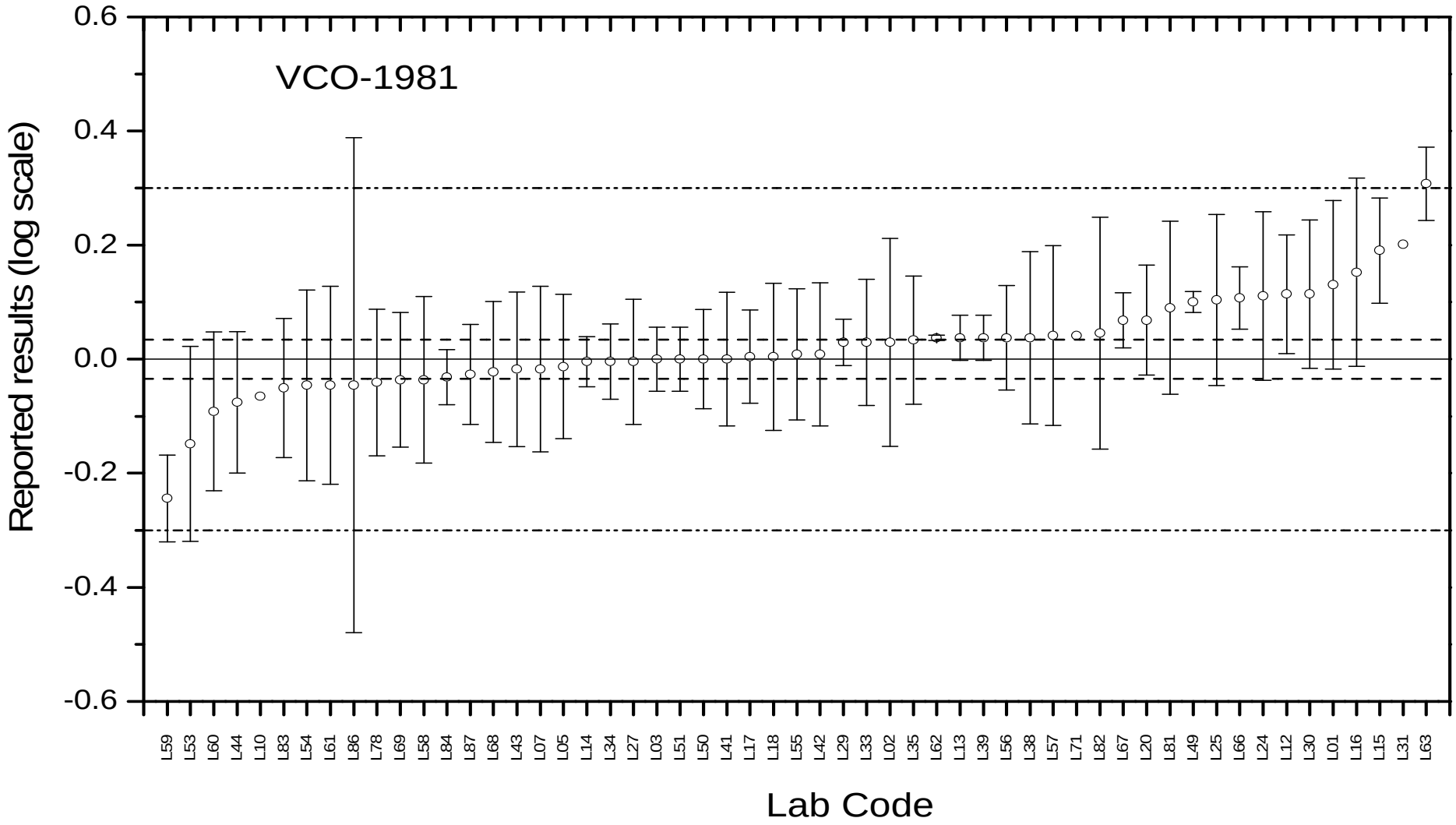


Figure A4.2. Laboratory results for maize event VCO-1981 in test item 2.

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



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