Determination of GM soybean event MON87708 in soy protein concentrate and GM maize event MON89034 in cornflakes

Report of the EURL GMFF proficiency test GMFF-24/02

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Contents

Abstract

Implementation of the European legislation on genetically modified organisms (GMOs) requires monitoring their presence in food and feed by analytical testing. The analytical tests are carried out by laboratories designated for official controls by the EU Member States. In line with Regulation (EU) 2017/625 on official controls, the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organises proficiency tests (PTs) to assess the uniform and reliable performance of these control laboratories. This report summarises the results of the PT "GMFF-24/02" for the determination of GMOs in soy protein concentrate and cornflakes. The organic soy protein concentrate was spiked with GM MON87708 soybean powder (T1) and the organic cornflakes were spiked with GM MON89034 maize powder (T2). Fifty-nine laboratories participated in the PT round, including 44 National Reference Laboratories (NRLs) from 22 EU Member States, 11 EU Official Control Laboratories (OCLs) and 4 OCLs from EU-neighbouring or EFTA countries. The evaluation of their analytical performance confirms that most laboratories are able to identify and accurately quantify GMOs in these food samples. An important lesson learned from this PT is the need to use the latest batch of certified reference materials (CRMs) for calibration or its conversion factor when using dPCR.

Acknowledgements

The 59 laboratories listed hereafter are kindly acknowledged for their participation in the PT.

Organisation	Country
Umweltbundesamt GmbH	Austria
Institute for Food Safety (AGES), Vienna	Austria
SCIENSANO	Belgium
Walloon Agricultural Research Center (CRA-W)	Belgium
Flanders Research Institute for Agriculture, Fisheries and Food (ILVO)	Belgium
National Centre of Public Health and Analyses (NCPHA), Sofia	Bulgaria
Laboratory of SGS Bulgaria	Bulgaria
Croatian Agency for Agriculture and Food (HAPIH), Centre for Seed and Seedlings	Croatia
Croatian Institute of Public Health (HZJZ)	Croatia
State General Laboratory (SGL)	Cyprus
Crop Research Institute	Czech Republic
Danish Veterinary and Food Administration (DVFA)	Denmark
Finnish Customs Laboratory (TULLI)	Finland
Finnish Food Authority	Finland
Service Commun des Laboratoires (SCL) - L67	France
Agence Nationale de SÉcurité Sanitaire de l'alimentation, de l'environnement et du travail (ANSES)	France
BioGEVES	France
AGROLAB LUFA GmbH	Germany
SGS Analytics Germany GmbH	Germany
Landeslabor Berlin-Brandenburg	Germany
LTZ Augustenberg	Germany
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe	Germany
Federal Office of Consumer Protection and Food Safety (BVL)	Germany
CVUA Freiburg	Germany
Institute for Hygiene and Environment	Germany
Landeslabor Schleswig-Holstein	Germany
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V (LALLF MV)	Germany
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer	Germany
LAVES - LVI Braunschweig/Hannover	Germany
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Germany
Thueringer Landesamt fuer Verbraucherschutz	Germany
Eurofins GeneScan GmbH	Germany
General Chemical State Laboratory (GCSL)	Greece
National Food Chain Safety Office (NEBIH), Food Chain Safety Laboratory Directorate	Hungary
Centro di Ricerca Difesa e Certificazione (CREA)	Italy
Institute of Food Safety, Animal Health and Environment (BIOR)	Latvia
National Food and Veterinary Risk Assessment Institute (NFVRAI)	Lithuania
Laboratoire National de Santé (LNS)	Luxemburg
Regional Laboratory of Genetically Modified Food	Poland
Plant Breeding and Acclimatization Institute - NRI	Poland
Wojewódzki Inspektorat Weterynarii w Opolu	Poland
National Veterinary Research Institute (PIWET)	Poland
Instituto Nacional de Investigação Agrária e Veterinária (INIAV)	Portugal
Institute for Diagnosis and Animal Health (IDSA)	Romania
Laboratorul Central pentru Calitatea Semințelor și a Materialului Săditor (LCCSMS)	Romania
A Bio Tech lab ltd	Serbia

Organisation	Country
SP Laboratorija A.D.	Serbia
State Veterinary and Food Institute (VFI) in Dolny Kubin	Slovakia
Central Control and Testing Institute of Agriculture (CCTIA) in Bratislava	Slovakia
Analitica Alimentaria, GmbH	Spain
Laboratorio Arbitral Agroalimentario (MAPA)	Spain
Dirección de Ganadería-SeLyC	Spain
Centro Nacional de Alimentación	Spain
Laboratorio Central de Veterinaria (CAN)	Spain
Agrolab Ibérica SLU	Spain
Laboratorio Agroalimentario de Navarra	Spain
Livsmedelsverket (Swedish Food Agency)	Sweden
Federal Institute of Metrology (METAS)	Switzerland
National Food Reference Laboratory	Turkey

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The report has been authorised for publication by Ursula Vincent, Head of the Food and Feed Compliance Unit (JRC.F.5).

Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised the proficiency test (PT) "GMFF-24/02" for the determination of GMOs in food products to support the implementation of Regulation (EU) 2017/625 [1]. This PT was open to National Reference Laboratories (NRLs) and official control laboratories (OCLs) and was managed in line with ISO 17043:2010 [2].

Two PT items were distributed to participants. PT item T1 consisted of an organic soy protein concentrate spiked with GM MON87708 soybean powder. PT item T2 consisted of organic cornflakes spiked with GM MON89034 maize powder. The laboratories were requested to identify the GM event and quantify its content in each PT item and to assess compliance with the EU GMO legislation.

Fifty-nine laboratories participated in the PT round, comprising 44 NRLs from 22 EU Member States, 11 EU OCLs, and 4 OCLs from EU-neighbouring or EFTA countries.

The first step in GMO analysis, following DNA extraction, is the qualitative identification of any GM event(s) present in the PT items. Most of the laboratories applied screening tests as a first-line strategy to exclude some events for further analysis. The outcome of the screening tests was evaluated and most of these results were found correct. In a second step, event-specific qualitative tests were applied to identify the specific GM events, followed by quantification using event-specific real-time PCR (qPCR) or digital PCR (dPCR) methods.

For the PT item T1, 50 laboratories (84.7 %) correctly identified GM event, while 6 laboratories did not test the event, 2 did not provide results, and 1 failed to detect the MON87708 soybean event. Similarly, for the PT item T2, 51 laboratories (86.4 %) correctly identified the GM event, 6 laboratories did not test the event, and 2 laboratories failed to detect the MON89034 maize event.

The quantitative results reported for the MON87708 event in T1 and the MON89034 event in T2 were evaluated using *z* or *z* prime (*z'*) and zeta (ζ) scores, in accordance with ISO 13528:2022 [3], without prior log transformation. The relative standard deviation for proficiency assessment (σ_{pt}) was set to 25 % of the assigned values, based on the experience acquired in previous PT rounds.

Most of the laboratories (46 out of 59) reported a quantitative result for MON87708 in T1, while 47 reported results for the MON89034 event in T2. Three and four laboratories provided two sets of results - obtained by qPCR and dPCR - for T1 and T2, respectively. The majority of the results (84 % for T1, 97.9 % for T2) were assessed as satisfactory based on *z(')* scores. For T1, five results were questionable and 3 were unsatisfactory, while for T2, two results were questionable. Most participants reported a realistic expanded measurement uncertainty with the corresponding coverage factor.

The results and technical details provided in the questionnaire were analysed to assess trends:

- Most dPCR results were lower compared to the assigned value for T1, but similar to the assigned value in T2;
- For quantification of the MON89034 event in T2, most laboratories who used an older batch of the CRM AOCS 0906-E (with higher GM copy number) obtained a result below the assigned range. Conversely, most laboratories that had used the recent CRM batch 0906-E2 reported a result within the assigned range;
- No clear correlation was observed between the DNA extraction method used and the reported results.

Participants were also asked to assess the compliance of the PT items against the applicable EU legislation on GMOs. While the majority of laboratories (over 70 %) provided compliance assessments of PT items T1 and T2 in accordance with their measurement ranges (result ± measurement uncertainties), only 43 % and 20 % of the laboratories correctly identified the items (T1 and T2, respectively) as non-compliant due to the absence of required labelling. For T2, this discrepancy was largely attributed to the use of an older CRM batch for calibration, which led to an underestimation of the GM content.

This PT round confirms that most NRLs and OCLs are able to monitor and quantify the mass fractions of GMOs in food samples. However, further improvement is needed in compliance assessment to align with the applicable EU GMO legislation. The PT further highlights the importance of using the most recent CRM batch for calibration of qPCR measurements or applying the conversion factor derived from this latest CRM batch to convert dPCR results from a copy number ratio into a GM mass fraction.

1 Introduction

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF), hosted by the Joint Research Centre of the European Commission, organised a proficiency testing (PT) round for the identification and quantification of GM soybean event MON87708 in an organic soy protein concentrate matrix and GM maize event MON89034 in organic cornflakes, to support Regulation (EU) 2017/625 on official controls [1].

This PT was agreed with the Directorate General for Health and Food Safety (DG SANTE) as part of the EURL GMFF annual work programme for 2023-2024, thus complying with the mandate set in Regulation (EU) 2017/625 [1]. The PT round was open to National Reference Laboratories under Regulations (EU) 2017/625 (NRL/625) and (EU) No 120/2014 (NRL/120) [4] and, under certain conditions, also to official control laboratories (OCLs).

Two PT items were prepared and dispatched to participants for analysis. Soy protein concentrate (PT item T1) and organic cornflakes (PT item T2) were selected to represent commercial food products that are routinely analysed for the presence of GMOs by control laboratories in the EU.

This report summarises the outcome of the PT.

2 Scope

The present PT aims to assess the performance of NRLs and OCLs in the determination of the mass fractions of GMOs in market-relevant food products and the assessment of the compliance of the products in relation to the applicable EU Regulations (EC) No 1829/2003 [5] and (EU) No 619/2011 [6].

The PT was mandatory for NRL/625, recommended for NRL/120, and open to OCLs (under certain conditions).

This PT, organised in line with ISO/IEC 17043:2010 [2], is identified as "GMFF-24/02".

3 Set up of the exercise

3.1 Quality assurance

The JRC Unit hosting the EURL GMFF is accredited according to:

ISO/IEC 17043:2010 (certificate number: BELAC 268-PT, proficiency test provider)

ISO/IEC 17025:2017 (certificate number: BELAC 268-TEST, for homogeneity, stability and characterisation tests)

The reported results were evaluated following the relevant administrative and logistic procedures.

3.2 Confidentiality

The procedures used for the organisation of PTs guarantee that the identity of the participants and the information provided by them are treated as confidential. The participants in this PT received a unique laboratory code used throughout this report. However, the laboratory codes of NRLs appointed in line with Regulation (EU) 2017/625 [1] may be disclosed to DG SANTE upon request for the purpose of an assessment of their (long-term) performance. Similarly, laboratory codes of appointed OCLs may be disclosed to their respective NRL upon request.

3.3 Time frame

Invitation letter (Annex 1), sent to NRLs and selected OCLs	14 May 2024
Registration deadline	24 May 2024
Sample dispatch	11 June 2024
Results deadline	2 August 2024

3.4 Distribution

Each participant received:

- One bottle of PT item T1 (soy protein concentrate), containing approx. 5 g of dry powder material;
- One bottle of PT item T2 (maize flakes), containing approx. 5 g of dry powder material;
- A "PT item accompanying letter" (Annex 2).

Samples were dispatched at room temperature. Participants were asked to check if the bottles were not damaged after transport and to store the PT items at + 4 °C.

3.5 Instructions to participants

Detailed instructions were given to the participants in the "Instructions letter" (Annex 3), sent by email on the day of the dispatch, and providing the individual laboratory code to be used by every participant when submitting the results obtained. Participants were allowed to register their laboratory twice for this PT round if they intended to submit two sets of results, obtained via both qPCR and dPCR methodologies.

The PT items were *"derived from products that are not declared as containing GM material"* and were described as follows:

- T1 is composed of soy protein concentrate, used e.g. to make protein shakes for body fitness.
- T2 consists of ground maize flakes (not roasted), used e.g. to add to breakfast cereals.

The testing laboratories were requested to check for the presence of GMOs and assess the compliance of the samples with the applicable GMO legislation.

Specific tasks for participants

PT Item 1 – Soy protein concentrate (food):

- Identify the single (quantifiable) GM soybean event;
- Quantify the GM soybean content and assess compliance of the product with the applicable GMO legislation.

PT Item 2 – Maize flakes (food):

- Identify the single (quantifiable) GM maize event;
- Quantify the GM maize content and assess compliance of the product with the applicable GMO legislation.

Participants were requested to apply their routine procedures for GMO testing. As the homogeneity study was conducted using 200 mg sample intake for both PT items, this amount was set as the recommended minimum sample intake.

When submitting their results, participants were instructed (i) to select the appropriate option (*i.e.* "not tested", "present", "absent" (default) for qualitative tests, or "m/m %" when entering a quantitative value); (ii) to report results with their measurement uncertainty and coverage factor *k*; and (iii) to select the technique used from a drop-down list.

Participants received an individual code to access the on-line interface for reporting their measurement results.

Participants were also asked to fill in an online EU Survey questionnaire, accessible with a provided password. The questionnaire was designed for reporting the outcome of any qualitative screening tests applied and to collect additional information related to the methods used by the laboratories when performing the measurements.

4 PT items

4.1 Preparation

PT item T1 consisted of a soy protein concentrate powder (ordered from Body & Fit) mixed with the 100 % GMO Certified Reference Material (CRM) AOCS 0311-A2 (MON87708) soybean powder. The CRM was purchased from The American Oil Chemist' Society (AOCS). The presence of soybean GM events in the soy protein concentrate was assessed using a GM soybean event-specific pre-spotted plate, PSP [7]. Only trace amounts of MON87708 (Cq~37) and 40-3-2 soy (Cq~40) were detected in the soy protein concentrate powder. However, this did not affect the selection of the matrix, as the MON87708 event was spiked into the matrix, and 40-3-2 was detected close to the cut-off value of the PSP.

The particle size (PSA) and water content of the soy protein concentrate and the MON87708 powders were measured. The powders were subsequently mixed and manually dispensed into 20 mL glass vials (ca. 5 g per vial) using a vibrating feeder and a balance. The vials were then sealed under an argon atmosphere. The argon was added using a process scale freeze dryer (Epsilon 2 100D, Martin Christ). Each vial was capped and labelled with the PT identifier and a unique vial number. The vials were stored at +4 °C prior to shipment. A total of 120 vials were produced. Further details on the processing can be found in Table 1.

Table 1. *Characteristics of the base materials used for the preparation of T1.*

Characteristic	**Non-GM Soybean**	**GM Soybean MON87708**
Type of base material	Soy protein concentrate	100 % MON87708 soybean CRM
Origin	Body & Fit	AOCS 0311-A2
Mixing equipment	Turbula T2 mixer (step 1) / DynaMIX CM-200	
Water content in g/100 g, mean ± *U* (*k*=2), with *n*=3	9.84 ± 1.40	7.35 ± 0.47
Particle diameter[1] in µm, mean ± *U* (*k*=2), with *n*=3	136.3 ± 8.4	778.5 ± 13.3
Mass used to prepare T1 (g) - Step 1	112.80	10.76
Mass used to prepare T1 (g) - Step 2	671.28	123.56 of step 1

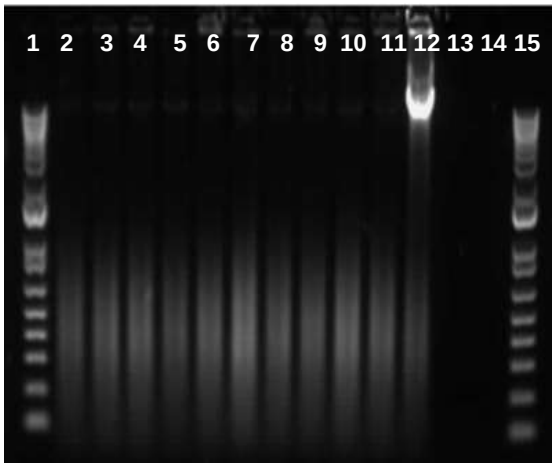
[1] Average equivalent sphere diameter of the X_{50} size class on the cumulative volume distribution curve
k: coverage factor; *U*: expanded measurement uncertainty

The DNA in the T1 material was extracted from samples of approximately 200 mg using a CTAB extraction method with Genomic-tip 20 purification. The quantity and quality of the extracted DNA were verified by UV spectrometry (Nanodrop) and fluorometry (PicoGreen). The extracted DNA exhibited high purity ($OD_{260/280}$~1.8 to 2.0, $OD_{260/230}$~2.1 to 2.4), and no PCR inhibition was detected during an inhibition test for the *le1* endogene using four serial dilutions (from 40 ng/µL to 0.09 ng/µL). The samples showed a smear on an agarose gel between 100-800 bp, indicating that the matrix was highly degraded (Figure 1,A). This was expected given that the production of soy protein concentrate includes heating and ethanol extraction steps [8]. This also explains the large difference in DNA quantification using Nanodrop and PicoGreen measurements (the latter measuring only double-stranded DNA) and the observation that low measurable DNA quantities by PicoGreen still resulted in strong amplification signals (low Cq values) in qPCR.

During the course of the characterisation measurements, the DNA extraction yield varied significantly between experiments but was consistent within the same experiment. Specifically, either a high DNA yield (around 35 ng/µL, as measured by PicoGreen) or a low DNA yield (around 9 ng/µL) was obtained. Using a TapeStation fragment analyser (Agilent Technologies), it was observed that the DNA samples with lower yields lacked the small, highly degraded DNA fragments present in the high-yield DNA samples (Figure 1,B). This loss of the smallest DNA fragments, likely originating from the (non-GM) soy protein matrix, led to an increased GM % that was consistent within each extraction experiment but approximately twice as high as the GM % measured on the high-yield DNA samples. A conclusive cause of these inconsistent results could not be determined. As the issue occurred during a specific time period (*i.e.* after the first extraction for homogeneity and in multiple extractions before the reporting deadline, but not anymore thereafter), it may be linked to a particular batch of Tip20 columns or buffers.

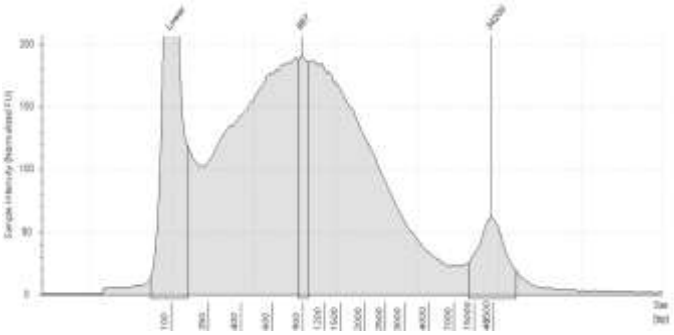
Figure 1. Fragment analysis of genomic DNA extracted from the T1 material

A - Agarose gel electrophoresis

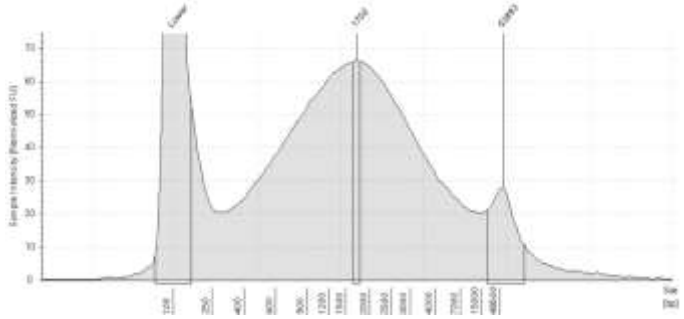


Lanes 2-11: T1;
Lane 12: CRM AOCS 0311-A2;
Lanes 13-14: blank samples;
Lane 1 & 15: Molecular marker, a 1 kb plus DNA ladder (Invitrogen, USA)

B – Fragment analysis on a TapeStation



Sample with high DNA yield – calculated average fragment size ~900 bp;



Sample with low DNA yield – calculated average fragment size ~1700 bp.

[Note the lower sample intensity on the Y scale in the bottom graph]

PT item T2 consisted of maize flakes commercially obtained from Pit & Pit that were milled into a powder and mixed with the 100 % GMO Certified Reference Material (CRM) AOCS 0906-E2 (MON89034) maize powder. The CRM was purchased from The American Oil Chemist' Society (AOCS). The presence of maize GM events in the maize flakes was assessed using a GM maize event-specific pre-spotted plate [9]. No GMO events were detected in the milled maize flakes powder.

The particle size (PSA) and water content of the maize flakes and the MON89034 powders were measured. To reduce the water content, the maize material was vacuum-dried prior to use. The powders were subsequently mixed and manually dispensed into 20 mL glass vials (ca. 5 g per vial) using a vibrating feeder and a balance. The vials were then sealed under an argon atmosphere. The argon was added using a process scale freeze dryer (Epsilon 2 100D, Martin Christ). Each vial was capped and labelled with the PT identifier and a unique vial number. The vials were stored at +4 °C prior to shipment. A total of 120 vials were produced. Further details on the processing can be found in Table 2.

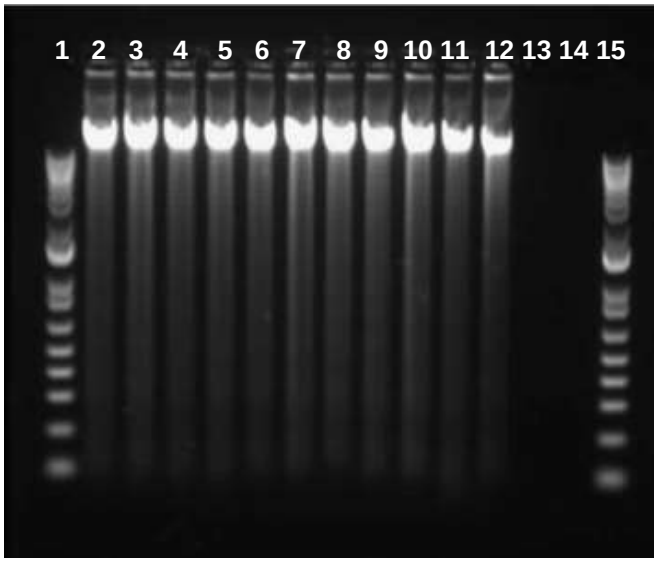
Table 2. *Characteristics of the base materials used for the preparation of T2.*

Characteristic	**Non-GM Maize**	**GM Maize MON89034**
Type of base material	Milled maize flakes	CRM AOCS 0906-E2
Origin	Pit & Pit	100 % MON89034 soybean
Grinding equipment	Cryo-grinding vibrating mill	Cryo-grinding vibrating mill
Mixing equipment	Turbula T2 mixer (step 1) / DynaMIX CM-200	
Water content in g/100 g, mean ± *U* (*k*=2), with *n*=3	2.58 ± 0.32	9.65 ± 1.21
Particle diameter[1] in µm, mean ± *U* (*k*=2), with *n*=3	72.9 ± 3.1	57.4 ± 4.3
Mass used to prepare T2 (g)	741.75	7.13

[1] Average equivalent sphere diameter of the X_{50} size class on the cumulative volume distribution curve
k: coverage factor; *U*: expanded measurement uncertainty

The DNA in the T2 material was extracted from samples of approximately 200 mg using a CTAB extraction method (without Genomic-tip 20 purification). The quantity and quality of the extracted DNA from the T2 material were verified by UV spectrometry, fluorometry and gel electrophoresis. Gel electrophoresis confirmed that the samples showed no major degradation (Figure 2). A selection of 10 DNA extracts, each at a concentration of 15 ng/µl, was tested for PCR inhibition using the maize reference gene *hmg* target (79 bp) with four serial dilutions (1:4, 1:16, 1:64, 1:256) and met the evaluation criteria (slope and ΔCq). For qPCR analysis, the samples were diluted to 15 ng/µl based on PicoGreen measurements to ensure that their Cq values fell within the calibration curves for both the endogene and transgene. All DNA extracts used for the homogeneity and stability studies (see section 4.2) contained sufficient amounts of DNA of suitable quality for PCR.

Figure 2. Agarose gel electrophoresis of genomic DNA extracted from the T2 material



Lanes 2-11: T2 material
Lane 12: CRM AOCS 0906-E2
Lanes 13-14: Blank samples
Lanes 1 & 15: Molecular marker, a 1 kb plus DNA ladder (Invitrogen, USA)

4.2 Homogeneity and stability

The measurements for the homogeneity and stability studies, as well as the statistical treatment of the data, were performed by the EURL GMFF (JRC Geel) for T1 and T2.

The detection method used for the measurements of T1 was the validated QT-EVE-GM-012 method, included in the GMOMETHODS database [10], which amplifies a 91 bp fragment targeting the 3' integration border region between the insert of soybean event MON87708 and the soybean host genome. The taxon-specific method targeted the soy lectin (*le1*) gene with a 74 bp amplicon (QT-TAX-GM-005).

The detection method used for the measurements of T2 was the validated QT-EVE-ZM-018 method in the GMOMETHODS database, which amplifies a 77 bp fragment targeting the 3' integration border region between the insert of maize event MON89034 and the maize host genome. The taxon-specific method used targets the maize high-mobility-group (*hmg*) gene, with an amplicon size of 79 bp (QT-TAX-ZM-002).

The assessment of **homogeneity** was performed after the processing and bottling of the PT items and before distribution to the participants. Ten items of T1 and T2 were randomly selected, and three independent replicates per unit were used for homogeneity analysis. DNA extraction was performed using the CTAB method, with genomic-tip 20 for T1 and without genomic-tip 20 purification for T2, using approximately 200 mg sample intakes. This amount was later set as the minimum sample intake.

The assessment of the homogeneity in T1 was performed on extracts with a low DNA yield, averaging 242 ng/µL as measured by Nanodrop and 9 ng/µL by Picogreen (see 4.1). One bottle was removed from analysis due to a technical error in one of the replicates.

Samples were diluted to 2.5 ng/µL to align fluorescence signals within the calibration curves for the endogene and transgene. The 100 m/m % soybean CRM AOCS 0311-A2 (40 ng/µL by PicoGreen) was used as the calibration standard. For the endogene, the calibration points were prepared by serial dilution from 100 % (40 ng/µL) to 50 %, 25 %, 10 %, 5 %, 1 %, and 0.5 % (0.2 ng/µL) using TE low buffer as a diluent. The MON87708 calibration curve included points at 10 % (4 ng/µL), 5 %, 1 %, 0.5 %, 0.1 %, 0.05 % and 0.02 % (0.008 ng/µL).

For T2, the DNA extracted from the PT items averaged 165 ng/µL by Nanodrop and 177 ng/µL by PicoGreen. The samples were diluted to 15 ng/µL for qPCR analysis. The 100 % maize CRM 0906-E22 (40 ng/µL PicoGreen) was used as the calibration standard, with calibration points for the endogene and transgene similar to those used for T1.

The qPCR results were evaluated according to ISO 13528:2022 [3]. The contribution from homogeneity (u_{hom}, Table 3 and 4) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated according to ISO Guide 35:2017 [11]. The T1 and T2 materials proved to be adequately homogeneous for the MON87708 soybean and MON89034 maize GM events, respectively (details in Annex 4.1).

For the **short-term stability** assessment, conducted in accordance with ISO13528:2022 [3], two test bottles (N=2) were stored during 1 week at 20 °C or 40 °C for T1, and at 40 °C for T2. The GM content was analysed in three replicates per bottle (n=3) and compared to samples stored at a reference temperature of -20 °C. qPCR measurements were performed under repeatability conditions, using T1 DNA extracts with a typically "low DNA yield". The results (Annex 4.2) showed no significant effect of storage at up to 40 °C on the stability of the test items compared to storage at -20 °C. Based on these findings, the test items were dispatched at room temperature.

The **long-term stability** of the PT items was assessed by qPCR, analysing the GM content in bottles (N=2, n=3) stored at +4 °C for 13 weeks, until after the reporting deadline. Results were compared to reference data obtained during the initial characterisation (time zero). For T1, DNA extracts with high DNA concentrations from the stability tests were compared to earlier extracts with similar high yields.

The data were evaluated against the storage time and a regression line was calculated. The slope of the regression line was tested for statistical significance (loss/increase due to storage). No significant trends were detected at a 95 % confidence level (Annex 4.2). This stability study confirmed that both PT items were adequately stable at +4 °C during the whole time period of the PT. The uncertainty contribution due to instability was set to zero (u_{stab}=0.

5 Assigned values and corresponding uncertainties

5.1 Assigned values

The homogeneity results reported by the EURL GMFF (JRC Geel) were also used to determine the assigned values for T1 and T2. Additionally, three external NRL laboratories were selected for the characterisation study based on their performance in previous EURL GMFF PT rounds (2019-2023) and their use of the appropriate methods within their ISO/IEC 17025 accreditation scope. Each laboratory was allowed to select its DNA extraction method, provided the extracted DNA met quality criteria ($OD_{260/280}$ and $OD_{260/230}$ ratios and PCR inhibition test for an endogene target) and was of sufficient quantity for qPCR. The laboratories analysed two bottles of each PT item, performed five independent DNA extractions from each bottle, and reported 10 results for each PT item.

The assigned values (x_{pt}) for the mass fraction of the GM events in the PT items were calculated as the mean of the reported results, in accordance with ISO 13528:2022 [3] (Tables 3 and 4).

Due to inconsistent results obtained by the EURL GMFF for the T1 material (see sections 4.1, 4.2), data from the JRC-Geel laboratory was excluded from value assignment. The assigned value for the mass fraction of the GM soybean event MON87708 in soy protein concentrate (T1) was determined solely based on the results from the three external expert laboratories (Table 3).

Similarly, the assigned value for the mass fraction of the GM maize event MON89034 in cornflakes (T2) was derived from results obtained by the EURL GMFF (JRC Geel) and two external laboratories. The data from one external laboratory was disregarded because of a technical issue.

In addition, Tables 3 and 4 present the robust means calculated from the results reported by the laboratories using Algorithm A [3]. This is further discussed in Section 7.3.1.

Table 3. *Assigned value (x_{pt}) and standard deviation for the proficiency assessment (σ_{pt}) for the MON87708 event in T1. All values (except last column) are expressed in m/m %.*

Laboratory	**DNA extraction method**	**Average ± *U* (*k*=2)**	x_{pt}	u_{char}	u_{hom}	$u(x_{pt})$ *k=1*	$U(x_{pt})$ *k=2*	σ_{pt}	$u(x_{pt})/\sigma_{pt}$
Lab 1	CTAB	1.17 ± 0.23	**1.304**	0.134 (10.3 %)	0.045 (3.4 %)	**0.141** (10.8 %)	0.282	0.326 (25 %)	0.43 (> 0.3)
Lab 2	Maxwell ® RSC PureFood GMO kit with Maxwell® 48 RSC instrument	1.57 ± 0.18							
Lab 3	CTAB + Maxwell MD automated extractor	1.17 ± 0.28							
Robust mean (Alg. A) ± MADe			**1.329**			**0.489**			

Table 4. *Assigned value (x_{pt}) and standard deviation for the proficiency assessment (σ_{pt}) for the MON89034 event in T2. All values (except last column) are expressed in m/m %.*

Laboratory	**DNA extraction method**	**Average ± *U* (*k*=2)**	x_{pt}	u_{char}	u_{hom}	$u(x_{pt})$ *k=1*	$U(x_{pt})$ *k=2*	σ'_{pt}	$u(x_{pt})/\sigma_{pt}$
EURL GMFF	CTAB	1.28 ± 0.15	**1.394**	0.074 (5.3 %)	0.061 (4.4 %)	**0.096** (6.9 %)	0.193	0.349 (25 %)	0.28 (< 0.3)
Lab 1	CTAB	1.54 ± 0.26							
Lab 3	CTAB + Maxwell MD automated extractor	1.37 ± 0.27							
Robust mean (Alg. A) ± MADe			**1.143**			**0.374**			

5.2 Associated uncertainties

The associated standard uncertainty of the assigned value ($u(x_{pt})$) was calculated following the law of uncertainty propagation, combining the standard measurement uncertainty of the characterisation (u_{char}) with the standard uncertainty contributions from homogeneity (u_{hom}) and stability (u_{stab}), in compliance with ISO 13528:2022 [3]:

$$u(x_{pt}) = \sqrt{u_{char}^2 + u_{hom}^2 + u_{stab}^2} \qquad \text{Eq. 1}$$

The uncertainty u_{char} is estimated as the standard error of the mean, according to ISO 13528:2022 [3]:

$$u_{char} = \frac{s}{\sqrt{p}} \qquad \text{Eq. 2}$$

where "*p*" is the number of datasets, while "s" is the standard deviation of the "*p*" dataset means.

5.3 Metrological traceability of the assigned value

The metrological traceability to the SI of the assigned values is proven by the following facts:

- only validated methods were used during the characterisation study;
- The calibrate balances used for weighing are maintained under ISO/IEC 17025 [12];
- all the values reported by the expert laboratories were traceable to the SI unit via the use of a common CRM with certified values traceable to the SI;
- satisfactory agreement of the reported results within their respective uncertainties.

5.4 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviation for PT assessment (σ_{pt}) was set to 25 % of the assigned value, based on the experience acquired in previous PT rounds.

6 Scores and evaluation criteria

Laboratory competence in qualitatively identifying a GM event in a PT item was evaluated. This information had to be selected from a drop-down menu (with options including absent [default], present, not tested or m/m %) when reporting the results through the JRC Multi Inter-Laboratory Comparison platform (MILC), as specified in the instructions letter. It is expected that all laboratories with the sample matrix and the GM event within their scope of analysis should be capable of identifying any GM event present in the PT items.

It is worth noting that, unlike other commercial GM-related PTs, the participants' reported data were not subjected to log10 transformation prior to the performance assessment [13]. The individual laboratory performance for the determination of the GM content was expressed in terms of z and ζ scores according to ISO 13528:2022 [3]:

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}} \qquad \text{Eq. 3}$$

$$\zeta = \frac{x_i - x_{pt}}{\sqrt{u^2(x_i) + u^2(x_{pt})}} \qquad \text{Eq. 4}$$

where: x_i is the measurement result reported by a participant;
$u(x_i)$ is the standard measurement uncertainty reported by a participant;
x_{pt} is the assigned value;
$u(x_{pt})$ is the standard measurement uncertainty of the assigned value;
σ_{pt} is the standard deviation for proficiency test assessment.

However, according to ISO 13528:2022 [3], when $u(x_{pt}) > 0.3\ \sigma_{pt}$ the uncertainty of the assigned value ($u(x_{pt})$) can be taken into account by expanding the denominator of the z score and calculating the z' score.

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u^2(x_{pt})}} \qquad \text{Eq. 5}$$

The interpretation of the z, z' and ζ performance scores is done according to ISO 13528:2022 [3]:

|score| ≤ 2.0	satisfactory performance	(green in Annex 6)
2.0 < |score| < 3.0	questionable performance	(yellow in Annex 6)
|score| ≥ 3.0	unsatisfactory performance	(red in Annex 6)

The ***z* (or z') scores** compare the participant's deviation from the assigned value with the standard deviation for proficiency test assessment (σ_{pt}) used as common quality criterion.

The ***ζ* scores** state whether the laboratory's result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value $u(x_{pt})$ and the measurement uncertainty as stated by the laboratory $u(x_i)$. 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 an inappropriate estimation of the concentration, or of its measurement uncertainty, or both.

The standard measurement uncertainty of the laboratory $u(x_i)$ was obtained by dividing the reported expanded measurement uncertainty by the reported coverage factor, *k*. When *k* was not specified, the reported expanded measurement uncertainty was considered by the PT coordinator as the half-width of a rectangular distribution; $u(x_i)$ was then calculated by dividing this half-width by √3, as recommended by Eurachem [14].

Uncertainty estimation is not trivial, therefore an additional assessment was provided to each laboratory reporting measurement uncertainty, indicating how reasonable their measurement uncertainty estimation has been. The relative standard measurement uncertainty was calculated based on the absolute values of the assigned values [$u_{rel}(x_{pt}) = 100*(u(x_{pt})/x_{pt})$] and of the reported values [$u_{rel}(x_i) = 100*(u(x_i)/x_i)$].

The relative standard measurement uncertainty from the laboratory $u_{rel}(x_i)$ is most likely to fall in a range between a minimum and a maximum allowed uncertainty (case "a": $u_{min,rel} \leq u_{rel}(x_i) \leq u_{max,rel}$). $u_{min,rel}$ is set to the standard uncertainties of the assigned values $u_{rel}(x_{pt})$. It is unlikely that a laboratory carrying out the analysis on a routine basis would determine the measurand with a smaller measurement uncertainty than the expert laboratories chosen to establish the assigned value (ISO 13528:2022 §7.6) or, if applicable, by formulation (ISO 13528:2022 §7.3) or than the certified measurement uncertainty associated with a certified reference material property value (ISO 13528:2022 §7.4). $u_{max,rel}$ is set to the standard deviation accepted for the PT assessment, σ_{pt} (expressed as a percentage of the assigned value). Consequently, case "a" becomes: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$.

If $u_{rel}(x_i)$ is smaller than $u_{rel}(x_{pt})$ (case "b") the laboratory may have underestimated its measurement uncertainty. Such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the measurement uncertainty associated with the assigned value also includes contributions for homogeneity and stability of the PT item. If those are large, relative measurement uncertainties smaller than $u_{rel}(x_{pt})$ are possible and plausible.

If $u_{rel}(x_i)$ is larger than $\sigma_{pt,\%}$ (case "c") the laboratory may have overestimated its measurement uncertainty. An evaluation of this statement can be made when looking at the difference between the reported value and the assigned value: if the difference is smaller than the expanded uncertainty $U(x_{pt})$ then overestimation is likely. If the difference is larger but x_i agrees with x_{pt} within their respective expanded measurement uncertainties, then the measurement uncertainty is properly assessed resulting in a satisfactory performance expressed as a ζ score, though the corresponding performance, expressed as a *z* score, may be questionable or unsatisfactory.

7 Evaluation of reported results

7.1 Participants

Forty-four NRLs and 15 OCLs registered to this PT round, resulting in a total of 59 participants (Table 5). NRLs responsible for managing official controls under Regulation (EU) 2017/625 constituted 56 % of the participants (33 NRL/625). All EU Member States, except Malta which has not yet appoint an NRL for GMO controls, contributed to this PT round. As mentioned in Section 5.1, the Dutch, Italian and Slovenian NRLs contributed to the characterisation of the two PT items. Furthermore, according to our knowledge, Estonia and Ireland designated BIOR in Latvia and Wageningen Food Safety Research (WFSR) in The Netherlands, respectively, as their NRL for GMO analysis. Likewise, AGES is acting as the NRL for Northern-Ireland.

Table 5. *Overview of participants in GMFF-24/02 by country and category.*

Country	Participants	NRL/625	NRL/120 (and not NRL/625)	OCL (not NRL)
Austria	2	2		
Belgium	3	3		
Bulgaria	2	2		
Croatia	2	2		
Cyprus	1	1		
Czech Republic	1	1		
Denmark	1	1		
Estonia (represented by BIOR, LV)				
Finland	2	1	1	
France	3	3		
Germany	15	1	9	5
Greece	1	1		
Hungary	1	1		
Ireland (represented by WFSR, NL)				
Italy	1*		1	
Latvia	1	1		
Lithuania	1	1		
Luxembourg	1	1		
Malta	0			
Netherlands	0*			
Northern-Ireland (represented by AGES, AT)				
Poland	4	3		1
Portugal	1	1		
Romania	2	1		1
Serbia	2			2
Slovakia	2	2		
Slovenia	0*			
Spain	7	3		4
Sweden	1	1		
Switzerland	1			1
Turkey	1			1
Total	**59**	**33 (56 %)**	**11 (19 %)**	**15 (25 %)**

* An NRL in this Member State contributed to the characterisation of the assigned values and, therefore, was not considered a participant in the PT.

7.2 Qualitative results

The first task requested from the participants was to identify the soybean or maize GM event present in the PT items T1 and T2, respectively.

The first step in GMO analysis of routine samples generally consists of the application of **screening methods** to identify the GMO elements and/or constructs that may be present or absent in the sample, thus reducing the number of event-specific methods to be applied in further analytical steps.

The following screening elements could have been positively scored (as determined by the GMO-MATRIX tool [15] :

- In T1, containing MON87708 soybean: tE9;
- In T2, containing MON89034 maize: p35S, tNOS, pFMV (and Cry1Ab, imperfect annealing).

The screening results reported by the laboratories are shown in Annex 5. When the elements mentioned above are tested by a laboratory and found to be absent the cell is shown in red, as they should have been detected if the corresponding screening method was applied. Some observations:

- The presence of traces of 40-3-2 soybean in T1 is confirmed by the EURL GMFF. Therefore, weak detection of p35S and tNOS could have occurred; however, these results were not scored. Eleven laboratories scored p35S as positive, and 12 laboratories scored tNOS as positive, while others reported negative results.
- In T1, laboratory L18 erroneously reported the presence of elements CTP2-CP4-EPSP and pFMV.
- In T2, L14 failed to detect the p35S and tNOS screening markers, and L09 erroneously reported the presence of CTP2-CP4-EPSP.
- The target Cry1Ab is predicted *in silico* (using the GMO-MATRIX tool) to exhibit imperfect annealing in MON89034. Additionally, it remains unclear whether the Cry1Ab/Ac method would yield a positive result (Annex 5). Consequently, the presence or absence of this marker was not scored. Four laboratories out of 19 reported a positive result.

The evaluation of the screening results showed that the large majority of the laboratories had evaluated the screening tests correctly. Only three laboratories had reported false positive or false negative results for some of the tests applied.

The outcome of the screening tests reduces the number of potential GM events to be assessed in the samples. This is then further confirmed in qualitative GM event identification tests. The results of this analysis had to be reported as 'present', 'absent' or 'not tested' for each of the GM events listed in the form, including all authorised events in the EU, as well as those with pending or expired (and still valid) authorisation. The qualitative GM event identification results are summarised in Table 6 and detailed in Annex 5.

In **T1**, 98 % of the laboratories (50 out of 51) who analysed the sample for the presence of the soybean event MON87708 successfully identified it; only one laboratory failed to detect the event. Six laboratories did not analyse the PT item. One laboratory failed to extract good-quality DNA and, therefore, reported that it had not analysed the sample, while another laboratory did not provide information.

For **T2,** 96 % of the laboratories (51 out of 53) who analysed the sample for the presence of the maize event MON89034 successfully identified it, while two laboratories failed to detect the event. Additionally, six laboratories did not test the event in the sample.

In conclusion, most of the laboratories that tested the sample and corresponding GM event demonstrated their capability to identify the correct GM event in the soy protein concentrate and maize flakes matrices.

Table 6. *Summary of the reported qualitative identification of the GM event in T1 and T2, expressed as number of laboratories (green=correct, red=wrong, orange=not tested).*

Sample analysed?	Outcome	PT item T1	PT item T2
		MON87708 soybean	MON89034 maize
Analysed	Detected	50	51
	Not detected	1 (L51)	2 (L08, L51)
	Not tested	6 (L03, L15, L22, L29, L42, L56)	6 (L03, L15, L22, L40, L42, L56)
Not analysed		L49[a]	-
Not specified if analysed or tested		L53	-

[a] L49 failed to extract good-quality DNA and, did not analysed the sample

7.3 Quantitative results

7.3.1 Performance

The number and type of quantitative results reported for T1 and T2 are shown in Table 7.

Table 7. *Summary of the quantitative results reported for the GM events in T1 and T2, expressed as number of laboratories.*

Reported quantitative result	PCR system	T1	T2
		MON87708 soybean	MON89034 maize
Absolute value	qPCR (only)	37	37
	dPCR (only)	6	6
	qPCR and dPCR	3	4
Truncated value	qPCR	2	3
No value		11	9
Total number of laboratories		59	59

Laboratory performance in quantifying the spiked GM event in T1 and T2 was assessed using *z*, *z'* and zeta (ζ) scores. Figure 3 summarises the laboratories' performance, while Annex 6 presents the scores per laboratory. In the annex, satisfactory performance is highlighted in green, questionable in yellow, and unsatisfactory in red. Cells were left uncoloured when the outcome could not be evaluated. The corresponding Kernel density plots (included in the main graphs) were obtained using the software available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [16].

The kernel density distribution obtained on the T1 appears unimodal and approximately normal (see graph in Annex 6). The robust mean determined by Alg.A [3] (1.33 ± 0.49 [k=1]) is in good agreement with the assigned value (1.30 ± 0.14 [k=1]) (Table 3). For T2, a bimodal distribution is observed (Annex 6) with the first mode around 0.91 m/m % and a second one around 1.40 m/m %. The robust mean (1.14 ± 0.37 [k=1]) in this case does not align with the assigned value (1.39 ± 0.10 [k=1]); however, the second mode closely matches with the assigned value.

When comparing the reported results with the information provided in the questionnaire, a clear explanation for the deviations emerges. Among the 29 quantitative results below the assigned range (x_{pt}-U_{pt} = 1.20 m/m %, indicated by the lower blue line in the graph in Annex 6), 22 were obtained using the older CRM batch AOCS 0906-E (shown with red diamonds on the graph in Annex 6) and only 3 with the batch 0906-E2 (green squares), which is currently available from AOCS (the CRM used for 4 other results was either not specified or different). Conversely, among the results above the lower limit of this range (1.20 m/m %), 18 were obtained with the most recent CRM 0906-E2 (or its conversion factor when using dPCR), while 2 with the older batch 0906-E (the CRM used for 2 other results was not disclosed).

Importantly, there is a significant difference in the GM copy numbers between both batches. The first batch (not available from AOCS anymore) was processed from hybrid seeds with a female GM donor (conversion factor CF = 0.58), while the second batch originated from a male GM donor (CF = 0.36). Calibrating qPCR results with the older CRM (which contains more GM copies) instead of the most recent CRM leads to lower values for a given sample. This also explains why the qPCR result of L54 (calibrated with the 0906-E CRM) was lower than their dPCR result (which correctly applied the correct CF of 0.36 established on 0906-E2). Additionally, L38 used a plasmid for calibration and the outdated CF of 0.58, resulting in an underestimated value of 0.8 m/m %.

This information regarding the official CRM for MON89034 was previously communicated to the NRLs during the EURL GMFF Workshop for NRLs in 2023. The key point communicated was: "*Male GM donor for the new batch 0906-E2 (CF = 0.36) replacing the first batch 0906-E (CF = 0.58) produced with a female GM donor*". The concluding message emphasised the importance of **regularly checking the list of the CFs, which are established on the most recent CRM, as changes to the CF may occur (e.g. with the renewed batch of maize MON89034)**.

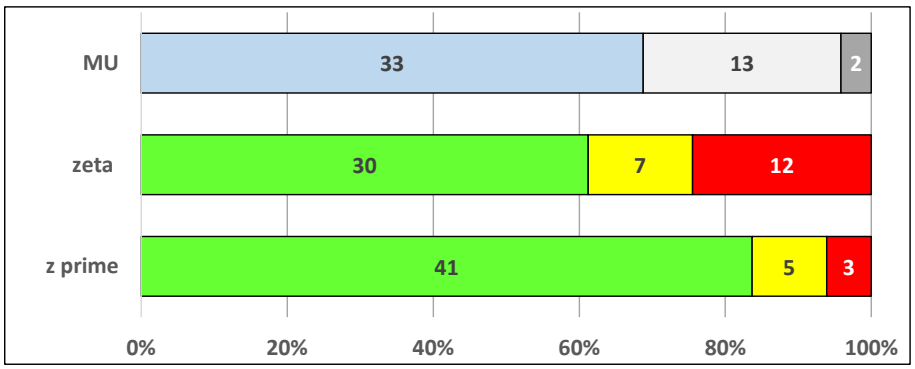
Performance scores were assigned to 49 results reported by 46 laboratories for T1, and to 51 results reported by 47 laboratories for T2. Three (T1) and four (T2) laboratories reported results obtained by both qPCR and dPCR, as indicated in Table 7. The vast majority of the results were considered satisfactory, as expressed by their *z* and *z'* scores (84 % for T1, 96 % for T2). For T1, three results were deemed unsatisfactory and 5 were flagged as questionable. For T2, only two results were flagged as questionable, both as a result of an underestimation of the GM content: L37 used the old CRM batch, L53 did not specify the CRM batch used. For the unsatisfactory results in T1, two laboratories overestimated the assigned value - L43 (overestimated by qPCR, though the *z'* score was satisfactory for dPCR) and L58 – while L40 underestimated it.

Expressed as zeta (ζ) score, significantly more unsatisfactory results were scored for T2 (17) compared to T1 (12). However, more questionable results were obtained for T1 (10) compared to T2 (7). This may be attributed to the likely underestimated measurement uncertainty reported by the participants.

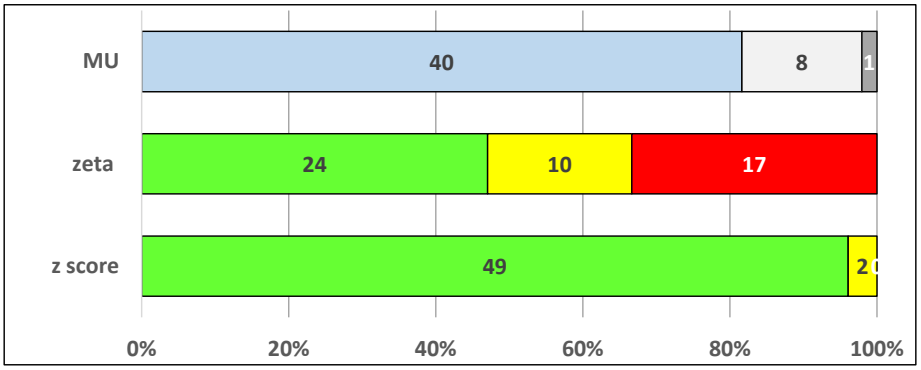
The unsatisfactory results in T1 were not unexpected, as T1 is a challenging protein-rich matrix with highly degraded DNA. The experimental findings demonstrated that degraded and fragmented DNA from the protein matrix could partially be lost during DNA extraction, resulting in inconsistent GM values (see sections 4.1, 4.2, and 5.1).

Figure 3. Overview of laboratory performance according to *z(')* and *ζ* scores.

A – Soybean event MON87708 in T1



B – Maize event MON89034 in T2



Satisfactory, questionable and unsatisfactory performance scores are indicated in green, yellow and red, respectively. Corresponding numbers of results are shown in the bars. Measurement uncertainty (MU) evaluated as follows: Case “a” (blue): $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$ Case "b" (light grey): $u_{rel}(x_i) < u_{rel}(x_{pt})$; Case "c" (grey): $u_{rel}(x_i) > \sigma_{pt,\%}$.

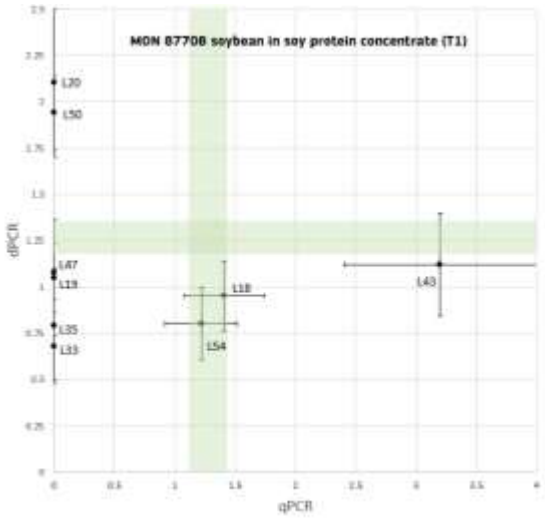
7.3.2 Comparison of results obtained by real-time PCR and digital PCR

Whereas most laboratories used real-time PCR (qPCR) for their measurements, several laboratories also reported digital PCR results (see Table 7). Three to four laboratories reported results obtained both by qPCR and by dPCR for one or both measurands. A comparison of the reported qPCR and dPCR results shows that, for MON87708 soybean in T1, most of the dPCR results are lower than the assigned value (Figure 4). In contrast, for MON89034 maize in T2, qPCR and dPCR results are generally comparable. Both observations - dPCR being lower than qPCR for T1 and similar values for T2 - were confirmed by the EURL GMFF. The reason for the discrepancy in T1 is unclear, although it appears that the degraded DNA from the soy protein matrix may be more efficiently amplified in dPCR, potentially increasing the proportion of the endogene.

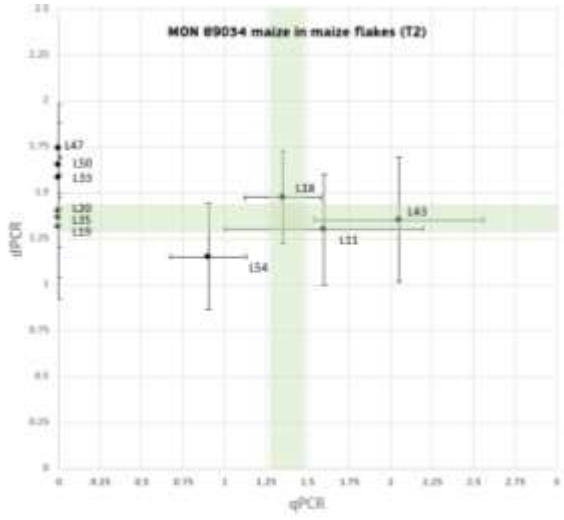
To convert the dPCR results (expressed as copy number ratio) into a corresponding GM mass fraction, most laboratories applied the conversion factor recommended by the EURL GMFF [17]. For MON87708 soybean, a factor of 1.03, established for the 100% CRM AOCS 0311-A2, was used. This value is consistent with the expected transgene/endogene ratio for a homozygous crop. In the case of MON89034 maize, which is hemizygous with a transgenic male parent, the established conversion factor for the 100% CRM AOCS 0906-E2 (with male GM donor) was 0.36. However, the previous batch of this CRM (0906-E, with a female GM donor) had a conversion factor of 0.58.

Figure 4. qPCR versus dPCR results (in m/m %)

A – Soybean event MON87708 in T1



B – Maize event MON89034 in T2



The green areas represent the assigned range (x_{pt} ± U).
dPCR results for which no corresponding qPCR result was reported are shown on the Y axis.

7.3.3 Truncated values

Two and three laboratories reported truncated (larger than) values for the measurands in T1 and T2, respectively. These values are considered plausible, but cannot be used for an evaluation of the compliance of the sample.

7.3.4 Measurement uncertainties

All laboratories that reported quantitative results for T1 and T2 provided an expanded measurement uncertainty and a coverage factor, except laboratory L57 (Annex 6). The majority of these laboratories (33 out of 48 for T1 and 40 out of 49 for T2) reported a realistic measurement uncertainty (Case "a" in Figure 3). However, 13 laboratories for T1 and 8 laboratories for T2 reported seemingly underestimated uncertainties (Case “b”). Only two laboratories reported slightly overestimated uncertainties (Case “c”) ranging from 28 % to 32 %. Laboratories L07 and L57 did not consistently provide their measurement uncertainties.

7.4 Compliance statement

Regulation (EC) No 1829/2003 [5] establishes a labelling threshold for food and feed products containing authorised GM material within the EU (0.9 m/m %), accounting for adventitious or technically unavoidable presence. Furthermore, Regulation (EU) No 619/2011 [6] introduces a minimum performance limit of 0.1 m/m % for detecting the accidental presence of GM material in feed, particularly those with pending or expired authorisation status. Member States of the European Union verify compliance with these limits during official controls on food and feed.

Laboratories were requested to provide a compliance statement for the T1 and T2 samples, in relation to the applicable EU legislation, *i.e.* Regulation (EC) No 1829/2003 [5] (for authorised GMOs in food and feed, labelling if > 0.9 m/m %) or Regulation (EU) No 619/2011 [6] (adventitious or technically unavoidable minimum presence of GM events listed in the EU Register allowed in feed if ≤ 0.1 m/m %).

The compliance statements provided for both the T1 and T2 samples were evaluated, and the outcome is summarised in Tables 8 and 9. While it is acknowledged that some testing laboratories may not routinely provide such statements to their Competent Authorities when reporting results, the majority of European laboratories are expected to be familiar with EU labelling regulations and capable of accurately interpreting their findings.

To evaluate compliance, the questionnaire included a stepwise guide. Laboratories were instructed to first determine whether the identified GM event(s) were authorised in the EU or fell under Regulation (EU) No 619/2011 for pending authorisations. Consequently, they were asked to report the (highest) result (x_i) and its expanded measurement uncertainty (U), calculate the "x – U" value, and finally evaluate whether the resulting value exceeded the threshold specified in the applicable Regulation.

As both PT items were for food use, **Regulation (EU) No 619/2011 does not apply**. Therefore, any compliance statement in line with this Regulation is wrong.

The invitation letter (Annex 1) explicitly states that the PT items are derived from products **not labelled as containing GMOs**.

The MON87708 soybean event present in **T1** is authorised in the EU; therefore, the reported range (result ± expanded uncertainty) should be compared against the labelling threshold of 0.9 % (m/m), as stipulated by Regulation (EC) No 1829/2003, assuming no unauthorised were identified. The following assumptions are taken into account:

- The content of MON87708 soybean in T1 (1.30 ± 0.28 m/m %, k = 2) is above the threshold, indicating that labelling of the product is required. Therefore, the **product is deemed not compliant** with the labelling requirements in Regulation (EC) No 1829/2003.

Out of 53 laboratories, 23 (43 %) correctly identified the PT item (T1) as non-compliant due to the absence of required labelling (highlighted in green in Table 8). Conversely, 19 laboratories (36 %) reported a lower MON87708 content in T1, observing "x – U" ≤ 0.9 m/m %, and thus considering the sample compliant with the labelling rules (highlighted in orange in Table 8). Additionally, L31 erroneously concluded non-compliance, while L58 incorrectly concluded compliance with Regulation (EC) No 1829/2003, as neither considered measurement uncertainty. Furthermore, L17, L40, L58 provided a compliance statements under Regulation (EU) No 619/2011, which does not apply to food products.

Interestingly, L18 reported conflicting compliance statement for their dPCR (compliant) and qPCR results (not compliant). This discrepancy raises the question of whether one of the methods might carry more legal weight than the other. While the qPCR method has been validated, the dPCR method is derived from the original reference method. As such, the result obtained with the validated qPCR method would likely prevail in Court.

Table 8. *Reported compliance statements for T1 (soy protein concentrate) based on the number of laboratories.*

Compliance Statement	***x - U***	**Number of Laboratories**	**Comment**
Compliant, because product does not require GMO labelling	≤ 0.9 m/m %	19[a]	Compliance statement is in line with measurement result, but not with assigned value ± *U*
	> 0.9 m/m %	1	L58 reported >0.9 %, but selected compliant
Not compliant, because product should have been labelled	> 0.9 m/m %	23[a]	
	≤ 0.9 m/m %	1	L31 reported < 0.9 %, but selected non-compliant
C < LLP - Compliant, under Reg. 619/2011 but ≤ 0.1 m/m %	≤ 0.1 m/m %	1	L40 This Regulation does not apply to food
NC>LLP - Not compliant, under Reg. 619/2011 and > 0.1 m/m %	> 0.1 m/m %	3	L17, L40, L58 This Regulation does not apply to food
CNC – Can Not Conclude or did not quantify		9	
Total number of laboratories with a compliance statement		53	

[a] L18: compliant based on dPCR result, non-compliant based on qPCR result.

For **T2,** a similar assessment was made. The MON89034 maize event present in T2 is authorised in the EU; therefore, the reported range (result ± expanded uncertainty) should be compared against the labelling threshold of 0.9 m/m %, as stipulated by the applicable Regulation (EC) No 1829/2003, assuming no unauthorised were identified in the EU. The following assumptions were considered:

- The content of MON89034 maize in T2 (1.33 ± 0.22 m/m %) is above the threshold, indicating that labelling of the product is required. Therefore, **the product is deemed not compliant** with the labelling requirements in Regulation (EC) No 1829/2003.

Out of the 54 laboratories, only 11 (20 %) correctly identified the PT item (T2) as non-compliant due to the absence of required labelling (highlighted in green in Table 9). Conversely, 26 laboratories (48 %) reported a lower MON89034 content in T2, observing "x – U" ≤ 0.9 m/m %, and thus considering the sample compliant with the labelling rules (highlighted in orange in Table 9). This was largely due to the use of the old CRM batch for calibration, resulting in a lower GM % (as discussed earlier). Additionally, L31 erroneously concluded non-compliance, even though "x – U" ≤ 0.9 m/m %. Furthermore, L17, L58 provided a compliance statements under Regulation (EU) No 619/2011, which does not apply to food product.

Interestingly, L43 reported conflicting compliance statement for their dPCR (compliant) and qPCR results (not compliant). The concern raised earlier applies here too.

Table 9. *Reported compliance statements for T2 (maize flakes) based on the number of laboratories.*

Compliance Statement	***x – U***	**Number of Laboratories**	**Comment**
Compliant, because product does not require GMO labelling	≤ 0.9 m/m %	26[a]	Compliance statement is in line with measurement result, but not with assigned value ± *U*
	> 0.9 m/m %	0	
Not compliant, because product should have been labelled	> 0.9 m/m %	11[a]	
	≤ 0.9 m/m %	1	L31 reported < 0.9 %, but selected non-compliant
C < LLP - Compliant, under Reg. 619/2011 but ≤ 0.1 m/m %	≤ 0.1 m/m %	0	
NC > LLP - Not compliant, under Reg. 619/2011 and > 0.1 m/m %, in feed	> 0.1 m/m %	2	This Regulation does not apply to food
CNC – Can Not Conclude or did not quantify		10	
Total number of laboratories with a compliance statement		54	

[a] L43: non-compliant based on qPCR result, compliant based on dPCR result

7.5 Questionnaire

The questionnaire was answered by all laboratories except 3 OCL and 1 NRL/625 (L02, L04, L51 and L53). As one participant provided separate answers for their qPCR and dPCR results the total number of answers received was 56 (from 55 laboratories). The results provide valuable information about the participating laboratories, their analysis strategy and the analytical approaches used. Detailed information is available in Annex 7, which summarises all experimental details and comments provided by the participants. Note that not all questions were answered by all participants, therefore the total number of answers per question is not always the same.

The outcome of the **screening methods** used for T1 and/or T2 is summarised in Annex 5 and discussed in section 7.2. Eight laboratories additionally analysed the presence of the GM soybean (T1) or GM maize (T2) events that were not covered by any screening methods by qualitative event-specific methods. Three laboratories also tested for the presence of the unauthorised events LY038 and VCO-1981 maize and the expired event MON863.

Ten laboratories used dPCR and six of them modified the primer and/or probe concentrations to achieve optimal resolution. All laboratories except one (L19) used the conversion factor recommended by the EURL GMFF. L19 applied a slightly different conversion factor determined in their laboratory.

The questionnaire summary provides details about the DNA extraction procedure used, including the lysis conditions (temperature, time, addition of proteinase K or RNase). When the DNA was measured both by spectrophotometer and a fluorescence method (measuring only double-stranded DNA) in T1 and T2, less DNA was generally measured with a fluorescence method, particularly for T1. This was expected in T1 because the

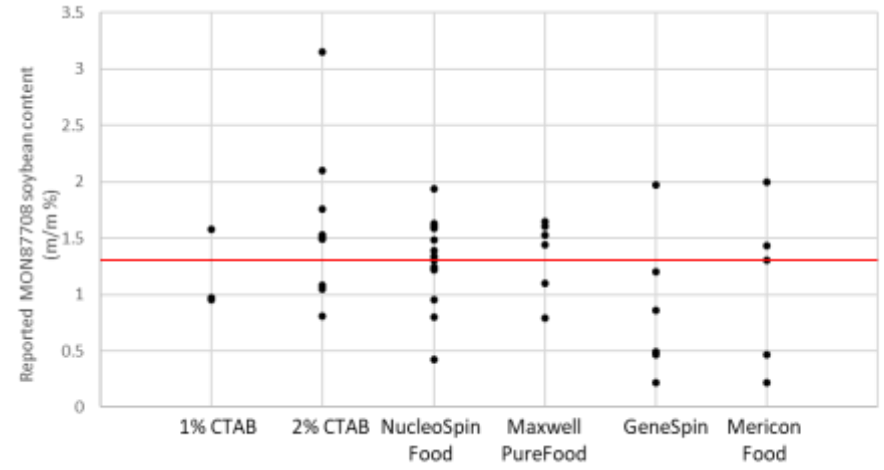
soy protein concentrate preparation was a processed powder with some of the DNA being degraded into single-stranded form during processing.

Of particular interest is to verify whether the DNA extraction method employed impacted the reported GM content. Different methods were used by the laboratories, mostly based on the use of the commercial kit NucleoSpin Food (14 results for T1 and T2), the use of 2 % CTAB (15), 1 % CTAB (5 results for T1 and 4 for T2) and other commercial kits such as Maxwell RSC PureFood (6), GeneSpin (6 for T1, 5 for T2) and Mericon Food (5).

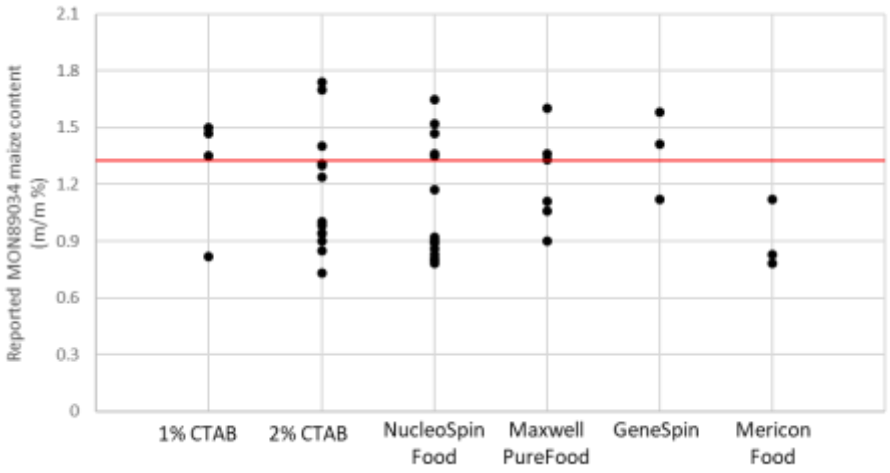
Comparison of the reported results from the most frequently used extraction methods reveals no significant effect of the specific DNA extraction method used on the results across laboratories (Figure 5).

Figure 5. Effect of DNA extraction method used on reported GM quantity for T1 (A) and T2 (B). The horizontal red line represents the assigned value.

A



B



8 Conclusions

The proficiency test GMFF-24/02 was organised to assess the analytical capabilities of EU NRLs and OCLs to analyse two food materials, soybean protein concentrate (T1) and maize flakes (T2), with the objective to identify the GM event present in the PT item and to determine the GM content.

All participants (except one or two) who tested the PT item or the specific GM event correctly identified the single GM event in T1 and T2. The vast majority of laboratories also reported a quantitative result, based on measurements using qPCR or dPCR, and most of these results were evaluated as satisfactory, expressed as z or *z'* scores.

Participants were also asked to assess the compliance of the PT items against the applicable EU legislation on GMOs. While the majority of laboratories (over 70 %) provided compliance assessments of PT items T1 and T2 in accordance with their measurement ranges (result ± measurement uncertainties), only 43 % and 20 % of the laboratories correctly identified the items (T1 and T2, respectively) as non-compliant due to the absence of required labelling.

This PT demonstrated that control laboratories are generally competent in assessing the presence of GMOs in food products on the EU market, thereby confirming their analytical capabilities to enforce the EU GMO Regulations [18]. This was particularly encouraging for the highly processed soy protein concentrate, from which highly degraded and fragmented DNA was extracted.

References

[1] Regulation (EU) No 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, Off. J. Eur. Union L 95: 1-142 (2017). https://europa.eu/!HnyGrd.

[2] ISO/IEC 17043:2010 (2010) Conformity assessment – General requirements for proficiency testing. International Organization for Standardization, Geneva, Switzerland.

[3] ISO 13528:2022 (2022) Statistical methods for use in proficiency testing by interlaboratory comparisons. International Organization for Standardization, Geneva, Switzerland.

[4] 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 (2014). https://europa.eu/!M4DBtc.

[5] 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 (2003). https://europa.eu/!C3JDkk.

[6] 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 (2011). https://europa.eu/!Krr7PR.

[7] 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 Systems for GMO detection. Food Anal Methods 2: 325-336. https://doi.org/10.1007/s12161-009-9093-0.

[8] Du, Y., Chen, F., Bu, G., Zhang, L. (2021) Distribution and degradation of DNA from non-genetically and genetically modified soybean (Roundup Ready): Impact of soybean protein concentrate and soybean protein isolate preparation. Food Chem. 335: 127582. https://doi.org/10.1016/j.foodchem.2020.127582.

[9] Rosa, S., Gatto, F., Angers-Loustau, A., Petrillo, M., Kreysa, J., Querci, M. (2016) Development and applicability of a ready-use PCR system for GMO screening. Food Chem. 201: 110-119. https://doi.org/10.1016/j.foodchem.2016.01.007.

[10] GMOMETHODS database. https://gmo-crl.jrc.ec.europa.eu/gmomethods/

[11] ISO Guide 35:2017 (2017) Reference materials - Guidance for characterization and assessment of homogeneity and stability. International Organization for Standardization, Geneva, Switzerland.

[12] ISO/IEC 17025:2017. Testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland.

[13] Broothaerts, W., Cordeiro, F., Corbisier, P., Robouch, P., Emons, H. (2020) Log-transformation of proficiency testing data on the content of genetically modified organisms in food and feed samples: Is it justified? Anal. Bioanal. Chem. 412, 1129–1136. https://doi.org/10.1007/s00216-019-02338-4.

[14] Ellison, S.L.R and 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.

[15] JRC GMO-Matrix compiling in silico PCR predictions for GMOs detection. https://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/.

[16] Analytical Methods Committee (2006) Representing data distributions with kernel density estimates. AMC Tech. Br. 4, 2. http://www.rsc.org/images/brief4_tcm18-25925.pdf.

[17] List of Conversion Factors (CF) provided by the EURL GMFF. https://gmo-crl.jrc.ec.europa.eu/doc/CF-CRM-values.pdf.

[18] Broothaerts, W., Cordeiro, F., Robouch, P., Emons, H. (2020) Ten years of proficiency testing reveals an improvement in the analytical performance of EU National Reference Laboratories for genetically modified food and feed. Food Control 114: 107237. https://doi.org/10.1016/j.foodcont.2020.107237.

List of abbreviations and symbols

AOCS	The American Oil Chemist' Society
bp	Base pairs
dPCR	Digital Polymerase Chain Reaction
DG SANTE	Directorate General for Health and Food Safety
EU	European Union
EURL	European Union Reference Laboratory
GMFF	Genetically Modified Food and Feed
GM(O)	Genetically modified (organism)
GUM	Guide for the Expression of Uncertainty in Measurement
ISO	International Organization for Standardization
JRC	Joint Research Centre
LOD	Limit of detection
LOQ	Limit of quantification
m/m %	GM mass fraction or mass per mass percentage
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PSP	Pre-spotted plate
PT	Proficiency Test
qPCR	Quantitative (real-time) Polymerase Chain Reaction
k	Coverage factor
σ_{pt}	Standard deviation for proficiency test assessment
$u(x_i)$	Standard measurement uncertainty reported by participant "i"
$u(x_{pt})$	Standard uncertainty of the assigned value
u_{char}	(Standard) uncertainty contribution due to characterisation
u_{hom}	(Standard) uncertainty contribution due to inhomogeneity
u_{stab}	(Standard) uncertainty contribution due to instability
$U(x_i)$	Expanded uncertainty reported by participant "i" with the coverage factor k
$U(x_{pt})$	Expanded uncertainty of the assigned value with the coverage factor k
x_i	Mean value reported by participant "i"
x_{pt}	Assigned value
z (or z')	z (or z') score
ζ	zeta score

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Annexes

Annex 1. Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food (Geel)
Food and Feed Compliance

Geel,14 May 2024
JRC.F.5/WB/bk/ARES(2024)3986338

FOR THE ATTENTION OF THE
NATIONAL REFERENCE LABORATORIES (NRLS) FOR GMOS
AND SELECTED NON-EU OFFICIAL LABORATORIES

Subject: Invitation to participate to the Proficiency Testing round "GMFF-24/02"

Dear laboratory representative,

On behalf of the EURL for GM Food and Feed (EURL GMFF), we would like to invite you to participate in the proficiency test (PT) "**Determination of GM soybean in soy protein concentrate (T1, food) and GM maize in maize flakes (T2, food)**" You will receive two ground test materials, processed at JRC-Geel. They are taken from products that are both not labelled as containing GMOs.

This PT round involves the following tasks:

For T1, you are requested to check for the presence of GM soybean, quantify the identified (single) GM event, and assess the compliance of the product with the applicable GMO legislation.

For T2, you are requested to check for the presence of GM maize, quantify the identified (single) GM event, and assess the compliance of the product with the applicable GMO legislation.

The PT fulfils the EURL GMFF mandate under Regulation (EU) 2017/625. Participation is free of charge.

Please register electronically by using the link below and following the instructions on screen (please do not use capital letters except for the first letter of a name):

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=3061

Once you have submitted your registration electronically, you will have to sign it, date it and send it to us by e-mail (JRC-EURL-GMFF-CT@ec.europa.eu). **If you intend to submit two sets of results, obtained by qPCR and dPCR, you have to register your laboratory twice and write this on the two reporting forms returned to us by e-mail.**

The deadline for registration is set to	**Friday 24 May 2024**
Test items will be dispatched around	11 June 2024
The deadline for submission of the results is	Friday 2 August 2024

If you represent an NRL under Regulation (EU) 2017/625, **please distribute this letter to any official laboratory within your network of EU official control laboratories** for which you deem its participation as relevant considering all or any of the requested tasks. These laboratories are allowed to register for this PT using the registration details provided in this letter.

The procedures used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, upon request, the lab codes of the NRLs that have been designated in line with Regulation (EU) 2017/625 will be disclosed to DG SANTE for (long-term) performance evaluation. Lab codes of appointed official laboratories may also be disclosed to their NRL upon request.

Do not hesitate to contact us (JRC-EURL-GMFF-CT@ec.europa.eu) if you have further questions.

Kind regards,

e-signed

Dr. Wim Broothaerts

PT Coordinator

Annex 2. PT item accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance

EURL
European Union Reference Laboratory
for GM Food & Feed

Geel, 11 June 2024

Subject: **GMFF-24/02, a proficiency test (PT) to determine the GM content in two test materials, Soy protein concentrate (T1) and Maize flakes (T2)**

Dear participant,

Thank you for participating in this PT round. Please find in this parcel two test materials, labelled T1, Soy protein concentrate (5 g, dry) and T2, Maize flakes (5 g, dry). Both samples were dispatched at room temperature.

Upon arrival, you should store the samples at approx. +4 °C.

Please check whether the bottles for T1 and T2 remained undamaged during transport. If damaged, we will promptly replace the test item (please send us a picture).

Before taking the samples for the analysis, please leave the bottle at room temperature for 15 minutes and mix well the powder inside the bottle.

Further instructions on this PT round, your individual lab code and the passcode for entering the results have been provided by email to the person that registered for this round.

Please, contact the functional mailbox JRC-EURL-GMFF-CT@ec.europa.eu if you have further questions.

Thank you for your collaboration.

Yours sincerely,

Wim Broothaerts
PT coordinator
European Union Reference Laboratory for GM Food and Feed

Annex 3. Instructions letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health and Food
Food and Feed Compliance

Geel, 12 June 2024
JRC.F.5/WB/mt ARES(2024) 24-050

«Firstname» «Surname» (**«LCode»**)
«Organisation»
«Address»
«Zip» «Town»
«Country»

Reporting website	https://web.jrc.ec.europa.eu/ilcReportingWeb.
EU login	For help, ask for the *Participant's guidelines*
Password	«Part_key»
Questionnaire	https://ec.europa.eu/eusurvey/runner/GMFF-24_02questionnaire
Password	GMFF2402

Subject: **Instructions for GMFF-24/02, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* soy protein concentrate and maize flakes**

Dear Dr «Surname»,

Thank you for participating in GMFF-24/02. In one of the following days you should receive two test materials, T1 and T2, each containing 5 g of ground sample. **The bottles should be stored at +4 ºC.**

Both samples are *derived from products that are not declared as containing GM material*.

- T1 is composed of soy protein concentrate, used e.g. to make protein shakes for body fitness.
- T2 consists of ground maize flakes (not roasted), used e.g. to add to breakfast cereals.

The testing laboratories are requested to check the presence of GMOs and assess the compliance of the samples with the applicable GMO legislation.

Specific tasks

Test Item 1 – Soy protein concentrate (food) (5 g dry weight):

- Identify the single (quantifiable) GM soybean event;
- Quantify the GM soybean content and assess compliance of the product with the applicable GMO legislation.

Test Item 2 –Maize flakes (food) (5 g dry weight):

- Identify the single (quantifiable) GM maize event;
- Quantify the GM maize content and assess compliance of the product with the applicable GMO legislation.

Participants are requested to apply their routine approaches for GMO testing. It is recommended to use a **minimum sample intake of 200 mg for your DNA extractions for**

T1 and T2, as homogeneity of the test items has been demonstrated using this amount of sample.

Before taking the samples for the analysis, please leave the bottle at room temperature for 15 minutes and **mix the powder** inside the bottle with a spoon or heavy shaking.

When **reporting your results**:

- The reporting form displays all GM soybean and GM maize events that are currently authorised in the EU or with a pending authorisation status; these are the events for which a test result is expected in this PT round;
- Under "Unit", the default setting indicates "absent" for all displayed GM events; please change this into m/m % if reporting a quantitative (or a smaller/larger than) result, or to "present" (or leave it as "absent") for reporting qualitative results (based on screening or event-specific analysis; there is no need to enter a zero if not reporting a quantitative value); make sure you do this for all GM events indicated, as these results will be evaluated in the PT report (e.g. if you indicated "absent" for an event that was actually present, the PT report will indicate that you failed to detect the event);
- Select the "=" (default) or "<" or ">" signs for reporting values;
- Report results with their expanded (absolute) uncertainty (*U*) and coverage factor *k* (only numbers are accepted in these fields, not text);
- Select the technique used (default is "no technique"). Note that selecting a technique automatically requires the entering of a numerical value under "result" otherwise an error message will be shown. Either enter no technique (leave the default indication) if no result in m/m % is reported, or enter a zero (i.e. absent) or </> "LOD" (in m/m %).

Do not forget to click the "validate and save" button and the "**Submit my results**" button. Check your results carefully before submission, since this is your final confirmation. After submitting your results on-line, you should **sign the completed report form and send a pdf copy to the EURL GMFF by e-mail** as a formal validation of the data introduced through MILC. Save a copy of this form for your own records.

After submission of your quantitative results, please go to the weblink https://ec.europa.eu/eusurvey/runner/GMFF-24_02Questionnaire, enter the password (see text box on top of this letter below address line), and answer the questions of the survey. This survey includes questions on the analytical approaches used, and requests a statement on compliance of the product to the EU legislation. Submit your answers to the survey on-line (no need to send them by e-mail).

Keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike. Be aware of the existence of an appeal procedure in case you disagree with your scores.

The deadline for submission of the results and the questionnaire is Friday 2 August 2024. It will not be possible to submit your results after the deadline.

The EURL GMFF will analyse all data received and publish a report indicating the performance of your laboratory for the identification and/or quantification of the GM events. You will receive a copy of the report by e-mail. In case of an unsatisfactory performance, the NRL participants will be requested to fill in a form indicating the root-cause analysis and providing evidence demonstrating the effectiveness of the correction actions implemented.

Further support may be provided in order to understand the problem and improve the analytical performance of your laboratory.

You should keep the test items at approximately -20 °C in order to voluntary repeat the analysis in case of an unsatisfactory performance. Please, dispose the test items thereafter.

Thank you for the collaboration in this PT. Please, contact the functional mailbox JRC-EURL-GMFF-CT@ec.europa.eu for all issues related to this PT round.

Yours sincerely,

e-signed

Wim Broothaerts

PT coordinator, European Union Reference Laboratory for GM Food and Feed

Annex 4. Homogeneity and stability results

4.1 Homogeneity

Homogeneity of MON87708 soybean in T1 (qPCR) – based on "low DNA yield" extractions

Item number[a]	Replicate 1	Replicate 2	*Replicate 3*
4	1.66	2.37	2.11
20	1.82	2.21	2.44
28	2.13	2.15	2.40
36	2.21	2.35	2.38
70	2.05	2.54	1.98
75	2.20	2.21	2.10
85	2.22	2.25	2.29
103	2.06	1.66	2.13
109	2.29	2.09	2.03
Mean	2.16		
$s_{x,\,rel}$	5.1 %		
$s_{w,\,rel}$	10.3 %		
$s_{s,\,rel}$	0 %		
u^*_{rel} **(=** u_{hom}**)**	**3.4 %**		
$\sigma_{pt,\,rel}$	25 %		
$0.3 * \sigma_{pt,\,rel}$	7.5 %		
$s_{s,\,rel} \leq 0.3^* \sigma_{pt,\,rel}$	YES		
Assessment	Passed		

[a] One bottle was removed from the analysis due to a technical outlier for one of the replicates

Homogeneity of MON89034 maize in T2 (qPCR)

Item number	Replicate 1	Replicate 2	*Replicate 3*
11	1.37	1.32	1.26
12	1.28	1.28	1.46
24	1.10	1.35	1.19
27	1.16	1.26	1.09
31	1.37	1.41	1.29
38	1.30	1.26	1.37
42	1.33	1.25	1.29
55	1.37	1.25	1.43
80	1.25	1.25	1.34
100	1.19	1.16	1.15
Mean	1.28		
$s_{x,\,rel}$	5.6 %		
$s_{w,\,rel}$	5.9 %		
$s_{s,\,rel}$ **(=** u_{hom}**)**	**4.4 %**		
u^*_{rel}	1.9 %		
$\sigma_{pt,\,rel}$	25 %		
$0.3 * \sigma_{pt,\,rel}$	7.5 %		
$s_{s,\,rel} \leq 0.3^* \sigma_{pt,\,rel}$	YES		
Assessment	Passed		

Where: σ_{pt} is the standard deviation for the PT assessment,
s_x is the standard deviation of the sample averages,
s_w is the within-sample standard deviation,
s_s is the between-sample standard deviation,
u^* is the conservative value for the uncertainty associated with heterogeneity, as defined in ISO Guide 35 [11].

All values are in m/m %.

4.2 Stability

In the tables below, the short-term and long-term stability was assessed according to ISO 13528:2022 [3]. All values shown are in m/m %.

Short-term stability of MON87708 soybean in T1 (qPCR) – based on "low DNA yield" samples

Days at 40 °C	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	4	1.66	2.37	2.11	2.10
	20	1.82	2.21	2.44	
7	25	2.14	2.43	2.08	2.28
	71	2.53	2.28	2.23	

Slope+2*SE = 0.066

Slope-2*SE = -0.016

Stability: passed

Long-term stability of MON87708 soybean in T1 (qPCR) – based on "high DNA yield" samples

Weeks at 4 °C	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	4	1.26	1.33	1.38	1.22
	20	1.09	1.17	1.10	
13	15	1.26	1.23	1.16	1.24
	21	1.25	1.07	1.48	

Slope+2*SE = 0.013

Slope-2*SE = -0.010

Stability: passed

Short-term stability of MON89034 maize in T2 (qPCR)

Days at 40 °C	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	11	1.37	1.32	1.26	1.33
	12	1.28	1.28	1.46	
7	9	1.18	1.49	1.21	1.36
	96	1.51	1.26	1.51	

Slope+2*SE = 0.025

Slope-2*SE = -0.016

Stability: passed

Long-term stability of MON89034 maize in T2 (qPCR)

Weeks at 4 °C	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	11	1.37	1.32	1.26	1.33
	12	1.28	1.28	1.46	
13	50	1.27	1.42	1.24	1.30
	87	1.29	1.26	1.33	

Slope+2*SE = 0.004

Slope-2*SE = -0.009

Stability: passed

Annex 5. Evaluation of the reported screening results

- "+" = present, "-" = absent, empty = not tested
- Red cells indicate that the screening result is not in line with the GM event present in the PT item;
- Whole grey rows = no screening results were reported for the PT item

Screening results reported for **T1**

Lab code	p35S[1]	tNOS[1]	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	CV127	305423
L01	-	-	-	-			-	-	-		-		-					-	-	-	-
L02																					
L03	+	+	-	-			-		-				-								
L04																					
L05	-	-		-			-	-	-					-						-	-
L06	+	+	-	-			-		-											-	-
L07	-	-	-	-			-		-				-							-	-
L08																				-	-
L09	+	+	-	-			-										+		-	-	-
L10	-	-					-							-						-	-
L11	-	-	-	-			-	-	-								+			-	-
L12	-	-	-														+			-	-
L13	-	-	-	-	-				-	-			-								
L14	+	+		-			-		-					-			+			-	-
L15	+	+	-	-													+				
L16	-	-	-					-												-	-
L17	-	-	-					-	-								+			-	-
L18	+	-	-				+[3]	-	+[3]							-		+		-	-
L19			-	-			-									-		+		-	-
L20	-	-	-	-			-	-	-								+			-	-
L21	-	-	-	-	-				-											-	-
L22	-	-	-	-			-		-				-							-	
L23	-	-	-	-	-															-	-
L24	-	-	-				-		-				-							-	-
L25	-	-	-	-			-	-	-								+			-	-

Lab code	p35S[1]	tNOS[1]	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	CV127	305423
L26	-	-	-				-	-												-	-
L27	-	+	-				-													-	-
L28	-	-	-	-			-	-									+			-	-
L29	-	-							-												
L30	-	-	-	-			-	-												-	-
L31	-	-	-				-													-	-
L32	-		-																	-	-
L33	-	-	-	-			-									-		+		-	-
L34	-	-	-					-	-											-	-
L35	-	-	-				-													-	
L36	+	+	-	-			-	-						-						-	-
L37	-	-	-				-	-									+			-	-
L38	-	-	-	-			-													-	-
L39	-	-	-				-	-	-								+			-	-
L40																				-	-
L41	+	+	-														+			-	-
L42	-	-																		-	
L43	-	-	-						-								+			-	-
L44			-				-													-	-
L45	-	-		-			-	-						-			+			-	-
L46	-	-	-				-										+			-	-
L47																				-	-
L48	-	-	-					-	-											-	-
L49	-	-	-	-			-						-	-						-	-
L50	-	-	-	-			-	-	-			-								-	-
L51																					
L52																				-	-
L53																					
L54	-	+	-					-	-											-	-
L55	-	-		-			-	-		-	-			-						-	-
L56	-	-	-														+				
L57	+	+																		-	-

Lab code	p35S[1]	tNOS[1]	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	CV127	305423
L58	+	+	-	-			-													-	-
L59	+	+	-														+				

[1] The presence of traces of 40-3-2 soybean in the soy protein concentrate matrix is confirmed; p35S and tNOS may, therefore, be detected as weakly positive; the reported results for these screening markers are not scored
[2] The presence of the *Agrobacterium* border sequences was not tested by the EURL GMFF and the reported results are, therefore, not scored
[3] Only traces detected

Screening results reported for **T2**

Lab code	p35S	tNOS	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac[1]	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat[1]	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	40278	LY038	VCO-1981
L01	+	+	-	-			-	-	+		-		-					-	-	-	-	-
L02																						
L03	+	+	-	-			-		+				-									
L04																						
L05	+	+		-			-	-	+					-						-	-	-
L06	+	+	-	-			-		+											-		
L07	+	+	-	-			-		+				-									
L08																				-	-	-
L09	+	+	-	-			+										-		-	-		
L10	+	+					-							-						-		
L11	+	+																		-		-
L12	+	+	-																	-		-
L13	+	+	-	-	-				+	-			-							-		-
L14	-	-		-			-		+					-			-			-	-	-
L15	+	+	-	-													-					
L16	+	+	-					-	+											-		
L17	+	+	-					-	+								-			-	-	-
L18	+	+	-				-	-	+									+		-		
L19	+	+	-	-			-									-		+		-	-	-
L20																				-	-	-

Lab code	p35S	tNOS	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac[1]	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat[1]	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	40278	LY038	VCO-1981
L21	+	+	-	-	-				+											-	-	-
L22	+	+	-	-					+				-									
L23	+	+	-	-	-															-	-	-
L24	+	+	-				-		+				-							-	-	-
L25	+	+	-	-			-	-	+								-			-		
L26	+	+	-	-			-	+												-		
L27	+	+	-	-			-											+		-	-	-
L28	+	+	-	-			-	-									-			-		-
L29	+	+							+													
L30	+	+	-	-				-	+											-		-
L31	+	+	-																	-	-	-
L32	+	+	-	-																-	-	-
L33	+	+	-	-			-		+							-		+		-	-	
L34	+	+	-					+	+											-		-
L35	+	+	-	-			-								-					-		-
L36	+	+	-	-			-	-						-						-	-	-
L37	+	+	-				-	+									-			-	-	-
L38	+	+	-	-			-													-		-
L39	+	+	-	-			-	-	+								-			-		-
L40																						
L41	+	+	-														-			-		
L42	+	+																				
L43	+	+	-						+											-		-
L44	+	+					-							-						-	-	-
L45	+	+		-			-	-						-	-					-	-	-
L46	+	+	-				-	-												-		
L47																				-	-	-
L48	+	+	-					+	+											-		
L49	+	+	-	-			-						-	-						-		-
L50	+	+	-	-			-	-	+			-								-		-
L51																						
L52																				-		

Lab code	p35S	tNOS	PAT	BAR	CP4-EPSPS	CTP-CP4-EPSPS	CTP2-CP4-EPSPS	Cry1 Ab/Ac[1]	pFMV	pNOS	pNOS-nptII	t35S	nptII	p35S-pat[1]	p35S-nptII	pCsVMV-pat	tE9	Agro border[2]	CaMV	40278	LY038	VCO-1981
L53																						
L54	+	+	-					-	+											-		
L55	+	+		-			-	-		-	-			-						-	-	-
L56	+	+	-														-			-		
L57	+	+							+											-		
L58	+	+	-	-			-													-		
L59	+	+	-														-					

Annex 6. Results and laboratory performance

- ID = GM event identification: D = detected, ND = not detected, NT = not tested, ? = no information provided
- The PT coordinator set the measurement uncertainty $u(x_i)$ to zero when no expanded uncertainty was reported and the k factor to 1.73 if not reported (or reported as zero – e.g. L57)
- Performance scores (z and ζ): satisfactory, questionable, unsatisfactory (blanc if no absolute value reported)
- Measurement uncertainty (MU): a: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$; b: $u_{rel}(x_i) < u_{rel}(x_{pt})$; c: $u_{rel}(x_i) > \sigma_{pt,\%}$

MON87708 soybean in T1

Evaluation parameters: x_{pt} = 1.304 ; $u(x_{pt})$ = 0.141 ; σ_{pt} = 0.326 (all values in m/m %)

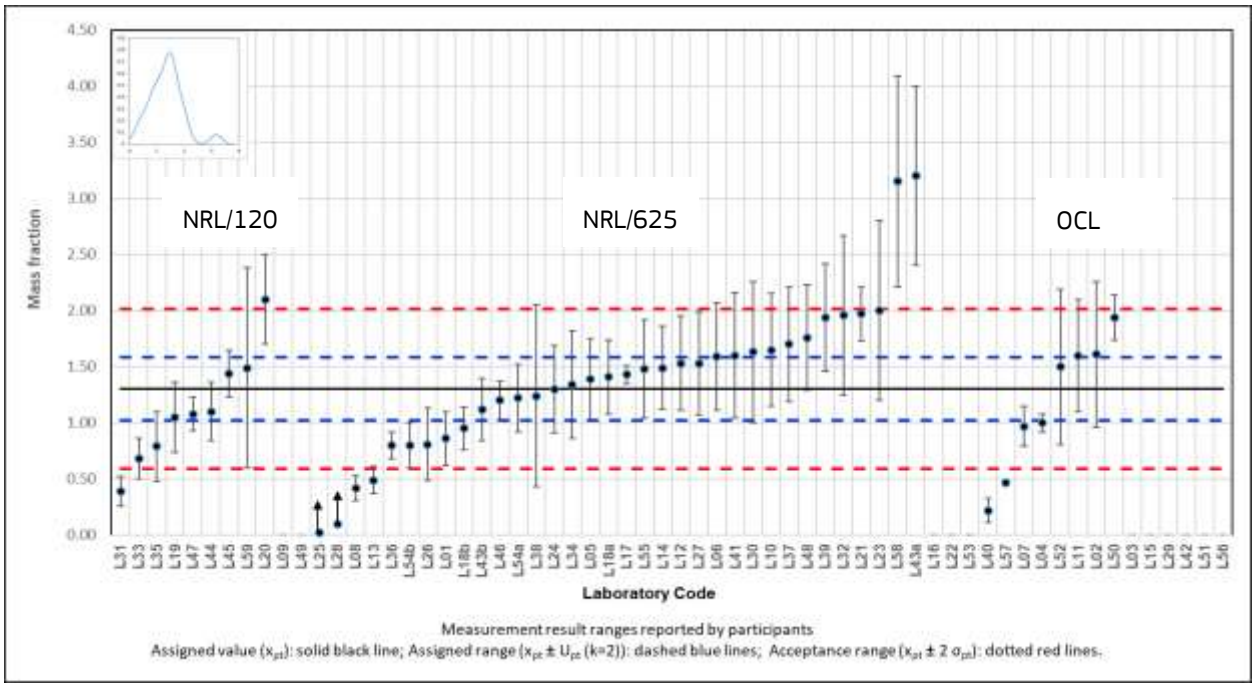
Type	LabCode	ID	x_i	±	k	Technique	z' score	ζ score	MU
NRL/625	L01	D	0.86	0.24	2	qPCR	-1.25	-2.40	a
OCL	L02	D	1.61	0.65	2	qPCR	0.86	0.86	a
OCL	L03	NT							
OCL	L04	D	1	0.08	2	qPCR	-0.86	-2.08	b
NRL/625	L05	D	1.39	0.36	2	qPCR	0.24	0.37	a
NRL/625	L06	D	1.59	0.48	2	qPCR	0.80	1.03	a
OCL	L07	D	0.97	0.18	2	qPCR	-0.94	-2.00	b
NRL/625	L08	D	0.42	0.11	2	qPCR	-2.49	-5.84	a
NRL/120	L09	D							
NRL/625	L10	D	1.65	0.5	1.73	qPCR	0.97	1.07	a
OCL	L11	D	1.6	0.5	2	qPCR	0.83	1.03	a
NRL/625	L12	D	1.53	0.42	2	qPCR	0.64	0.89	a
NRL/625	L13	D	0.49	0.12	2	qPCR	-2.29	-5.31	a
NRL/625	L14	D	1.49	0.3725	2	qPCR	0.52	0.79	a
OCL	L15	NT							
NRL/625	L16	D							
NRL/625	L17	D	1.43	0.08	2	qPCR	0.35	0.86	b
NRL/625	L18a	D	1.41	0.33	2.31	qPCR	0.30	0.53	b
NRL/625	L18b	D	0.95	0.19	2.78	dPCR	-1.00	-2.26	b
NRL/120	L19	D	1.05	0.31	2	dPCR	-0.72	-1.21	a
NRL/120	L20	D	2.1	0.4	2	dPCR	2.24	3.25	b
NRL/625	L21	D	1.97	0.24	2	qPCR	1.87	3.59	b
NRL/625	L22	NT							
NRL/625	L23	D	2	0.8	2	qPCR	1.96	1.64	a
NRL/625	L24	D	1.3	0.39	2	qPCR	-0.01	-0.02	a
NRL/625	L25	D	> 0.025			qPCR			
NRL/625	L26	D	0.81	0.32	2	qPCR	-1.39	-2.32	a
NRL/625	L27	D	1.53	0.46	2	qPCR	0.64	0.84	a
NRL/625	L28	D	> 0.1			qPCR			
OCL	L29	NT							
NRL/625	L30	D	1.63	0.63	2	qPCR	0.92	0.94	a
NRL/120	L31	D	0.39	0.13	2	qPCR	-2.57	-5.89	a
NRL/625	L32	D	1.957	0.71	1.73	qPCR	1.84	1.50	a
NRL/120	L33	D	0.68	0.18	2.36	dPCR	-1.76	-3.89	a
NRL/625	L34	D	1.34	0.48	2	qPCR	0.10	0.13	a
NRL/120	L35	D	0.79	0.31	2	dPCR	-1.45	-2.45	a
NRL/625	L36	D	0.8	0.12	2	qPCR	-1.42	-3.29	b

Type	LabCode	ID	x_i	±	k	Technique	z' score	ζ score	MU
NRL/625	L37	D	1.7	0.51	2	qPCR	1.11	1.36	a
NRL/625	L38	D	1.24	0.81	2	qPCR	-0.18	-0.15	c
NRL/625	L39	D	1.94	0.48	2	qPCR	1.79	2.28	a
OCL	L40	D	0.22	0.11	2	qPCR	-3.05	-7.16	a
NRL/625	L41	D	1.6	0.56	2	qPCR	0.83	0.94	a
OCL	L42	NT							
NRL/625	L43a	D	3.2	0.8	2	qPCR	5.34	4.47	a
NRL/625	L43b	D	1.12	0.28	2	dPCR	-0.52	-0.93	a
NRL/120	L44	D	1.1	0.26	3.18	qPCR	-0.58	-1.25	b
NRL/120	L45	D	1.44	0.21	2.57	qPCR	0.38	0.83	b
NRL/625	L46	D	1.2	0.17	2	qPCR	-0.29	-0.63	b
NRL/120	L47	D	1.08	0.15	2.78	dPCR	-0.63	-1.49	b
NRL/625	L48	D	1.76	0.47	2	qPCR	1.28	1.66	a
NRL/120	L49	?							
OCL	L50	D	1.94	0.2	2	dPCR	1.79	3.68	b
OCL	L51	ND							
OCL	L52	D	1.5	0.69	2	qPCR	0.55	0.52	a
NRL/625	L53	?							
NRL/625	L54a	D	1.22	0.3	2	qPCR	-0.24	-0.41	a
NRL/625	L54b	D	0.8	0.2	2	dPCR	-1.42	-2.92	a
NRL/625	L55	D	1.48	0.44	2	qPCR	0.49	0.67	a
OCL	L56	NT							
OCL	L57	D	0.47	0	1.73	qPCR	-2.35	-5.91	NP
NRL/625	L58	D	3.15	0.94	2	qPCR	5.19	3.76	a
NRL/120	L59	D	1.49	0.89	2	qPCR	0.52	0.40	c

GMFF-24/02: MON87708 in T1

x_{pt} = 1.30 **u(x_{pt}) =** 0.14 *(k=1)* **σ_{pt} =** 0.33 (in g/g)

σ'_{pt} = 0.36 **z prime**



MON89034 maize in T2

Evaluation parameters: x_{pt} = 1.394 ; $u(x_{pt})$ = 0.096 ; σ_{pt} = 0.349 (all values in m/m %)

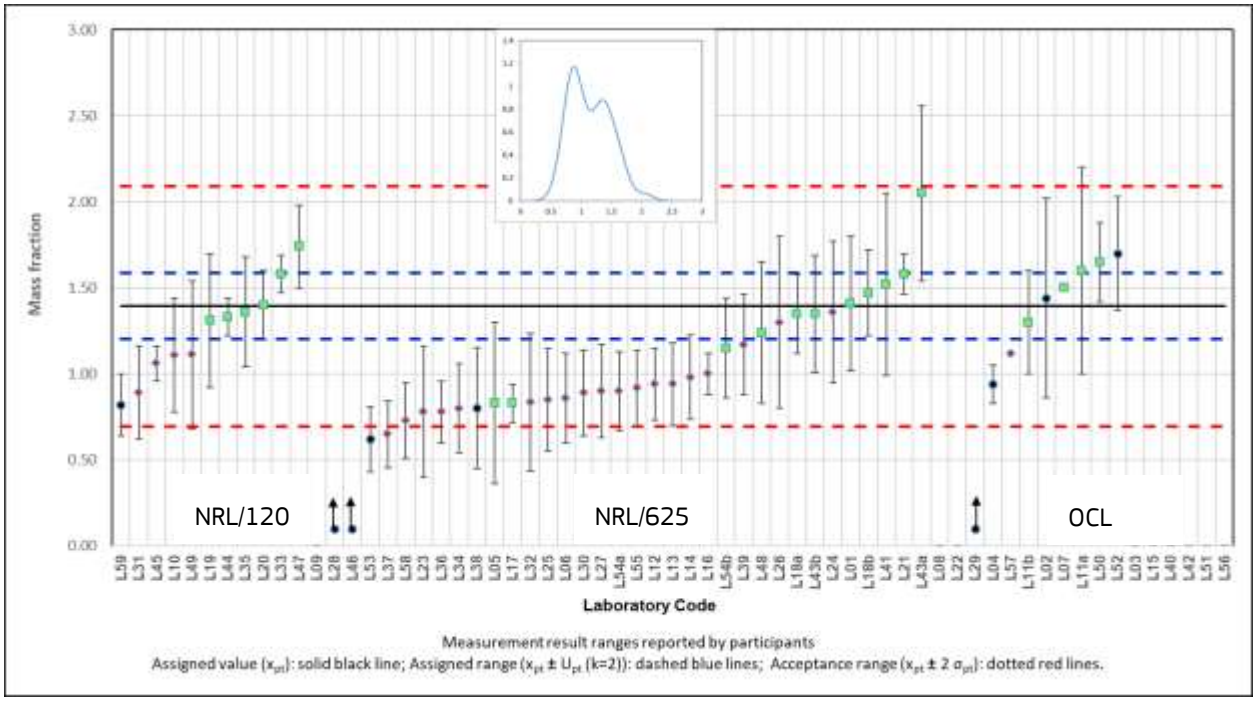
Type	LabCode	ID	x_i	±	k	Technique	z score	ζ score	MU
NRL/625	L01	D	1.41	0.39	2	qPCR	0.05	0.07	a
OCL	L02	D	1.44	0.58	2	qPCR	0.13	0.15	a
OCL	L03	NT							
OCL	L04	D	0.94	0.11	2	qPCR	-1.30	-4.09	b
NRL/625	L05	D	0.83	0.47	2	qPCR	-1.62	-2.22	c
NRL/625	L06	D	0.86	0.26	2	qPCR	-1.53	-3.30	a
OCL	L07	D	1.5	0	1.73	qPCR	0.30	1.10	NP
NRL/625	L08	ND							
NRL/120	L09	D							
NRL/120	L10	D	1.11	0.33	1.73	qPCR	-0.82	-1.33	a
OCL	L11a	D	1.6	0.6	2	qPCR	0.59	0.65	a
OCL	L11b	D	1.3	0.3	1.73	dPCR	-0.27	-0.47	a
NRL/625	L12	D	0.94	0.21	2	qPCR	-1.30	-3.18	a
NRL/625	L13	D	0.94	0.24	2	qPCR	-1.30	-2.95	a
NRL/625	L14	D	0.98	0.245	2	qPCR	-1.19	-2.66	a
OCL	L15	NT							
NRL/625	L16	D	1	0.12	2	qPCR	-1.13	-3.47	b
NRL/625	L17	D	0.83	0.11	2	qPCR	-1.62	-5.08	b
NRL/625	L18a	D	1.35	0.23	2	qPCR	-0.13	-0.29	a
NRL/625	L18b	D	1.47	0.25	2.14	dPCR	0.22	0.50	a
NRL/120	L19	D	1.31	0.39	2	dPCR	-0.24	-0.39	a
NRL/120	L20	D	1.4	0.2	2	dPCR	0.02	0.04	a
NRL/625	L21	D	1.58	0.12	2	qPCR	0.53	1.64	b
NRL/625	L22	NT							
NRL/625	L23	D	0.78	0.38	2	qPCR	-1.76	-2.88	a
NRL/625	L24	D	1.36	0.41	2	qPCR	-0.10	-0.15	a
NRL/625	L25	D	0.85	0.3	2	qPCR	-1.56	-3.05	a
NRL/625	L26	D	1.3	0.5	2	qPCR	-0.27	-0.35	a
NRL/625	L27	D	0.9	0.27	2	qPCR	-1.42	-2.98	a
NRL/625	L28	D	> 0.1						
OCL	L29	D	> 0.1			qPCR			
NRL/625	L30	D	0.89	0.25	2	qPCR	-1.45	-3.19	a
NRL/120	L31	D	0.89	0.27	2	qPCR	-1.45	-3.04	a
NRL/625	L32	D	0.835	0.4	2	qPCR	-1.60	-2.52	a
NRL/120	L33	D	1.58	0.11	2.36	dPCR	0.53	1.74	b
NRL/625	L34	D	0.8	0.26	2	qPCR	-1.70	-3.67	a
NRL/120	L35	D	1.36	0.32	2	dPCR	-0.10	-0.18	a
NRL/625	L36	D	0.78	0.18	2	qPCR	-1.76	-4.65	a
NRL/625	L37	D	0.65	0.195	2	qPCR	-2.13	-5.43	a
NRL/625	L38	D	0.8	0.352	2	qPCR	-1.70	-2.96	a
NRL/625	L39	D	1.17	0.29	2	qPCR	-0.64	-1.29	a
OCL	L40	NT							
NRL/625	L41	D	1.52	0.53	2	qPCR	0.36	0.45	a
OCL	L42	NT							

Type	LabCode	ID	x_i	±	k	Technique	z score	ζ score	MU
NRL/625	L43a	D	2.05	0.51	2	qPCR	1.88	2.41	a
NRL/625	L43b	D	1.35	0.34	2	dPCR	-0.13	-0.23	a
NRL/120	L44	D	1.33	0.11	3.18	qPCR	-0.18	-0.63	b
NRL/120	L45	D	1.06	0.1	2.57	qPCR	-0.96	-3.21	b
NRL/625	L46	D	> 0.1			qPCR			
NRL/120	L47	D	1.74	0.24	3.18	dPCR	0.99	2.82	b
NRL/625	L48	D	1.24	0.41	2	qPCR	-0.44	-0.68	a
NRL/120	L49	D	1.112	0.43	2	qPCR	-0.81	-1.20	a
OCL	L50	D	1.65	0.23	2	dPCR	0.73	1.70	a
OCL	L51	ND							
OCL	L52	D	1.7	0.33	2	qPCR	0.88	1.60	a
NRL/625	L53	D	0.62	0.19	2	qPCR	-2.22	-5.72	a
NRL/625	L54a	D	0.9	0.23	2	qPCR	-1.42	-3.29	a
NRL/625	L54b	D	1.15	0.29	2	dPCR	-0.70	-1.40	a
NRL/625	L55	D	0.92	0.22	2	qPCR	-1.36	-3.24	a
OCL	L56	NT							
OCL	L57	D	1.12			qPCR	-0.79	-2.84	NP
NRL/625	L58	D	0.73	0.22	2	qPCR	-1.91	-4.54	a
NRL/120	L59	D	0.82	0.18	2	qPCR	-1.65	-4.35	a

GMFF-24/02: MON89034 in T2

x_{pt} = 1.39 **u(x_{pt}) =** 0.10 *(k=1)* σ_{pt} = 0.35 (in g/g)

z score



Note: results obtained with CRM 0906-E (older batch) are shown with red markers, those obtained with CRM 0906-E2 (or its CF) with green markers.

Annex 7. Results of the questionnaire

The answers to the questionnaire are presented in the tables below. Note that in some cases only the most informative answers to open questions are shown or a summary of the answers is provided. As some laboratories reported both qPCR and dPCR results, the numbers shown refer to the number of answers, not the number of laboratories.

Please select which test items were analysed by your laboratory (Note: if you select "yes" to at least one of the test items, several further questions will pop up; if you select "no" for both test items, no further questions will be shown).

Answer	T1	T2
Yes	55	56
No	1	0
No Answer	4	4

Does your scope of accreditation under ISO/IEC 17025 covers DNA extraction:

Answer	From a soy protein concentrate matrix (T1)	From a maize flakes (food) matrix (T2)
Yes	47	47
No	5	7
Not clear	3	1
Not applicable	1	1
No answer	0	0

Select the sample intake (mg powder) per replicate used for DNA extraction (closest mass)

Test item	<100 mg	100 mg	150 mg	200 mg	300 mg	400 mg	500 mg	>500 mg
T1	1	2	0	38	3	1	3	11
T2	1	0	1	38	4	1	2	9

Select the DNA extraction method and any additional purification method(s) used for T1 and T2

DNA extraction method	T1	T2
CTAB method with 1% CTAB in lysis buffer	4	5
CTAB method with 2% CTAB in lysis buffer	15	15
Maxwell RSC PureFood GMO and authentication kit	6	6
Maxwell RSC/LEV Plant DNA kit	0	0
NucleoSpin Food	15	15
NucleoSpin Plant	1	1
NucleoMag DNA Food	3	2
GeneSpin	6	5
Qiagen DNeasy Plant	1	1
Qiagen DNeasy Mericon Food	5	5
Qiagen Blood and Tissue kit	1	1
Biotecon Foodproof	1	2
SDS	0	0
Speedtools Food DNA extraction kit (Biotools)	1	1
Eurofins DNAExtractor cleaning column	0	0
Promega Wizard DNA clean-up resin	1	1
Qiagen QIAQuick	2	2
Qiagen Genomic-Tip 20/G	0	0
NucleoSpin gDNA clean-up	0	0
Zymo OneStep PCR inhibitor Removal kit	0	0
Qiaex II purification kit	1	1

Indicate how the lysis step was performed during DNA extraction

Lysis condition	Detail	T1	T2
Denaturating agent (CTAB, SDS, guanidine-HCl, ...)	CTAB	25	27
	SDS, guanidine-HCl	5	5
	Lysis (CF) buffer from NucleoSpin Food kit	9	9
	Other	6	6
Incubation temperature (Celsius)	50-55 °C	2	2
	60 °C	8	8
	65 °C	42	41

	80 °C	1	1
Incubation time (min)	30-45 min	22	22
	60 min	13	11
	75-90 min	7	7
	120-150 min	5	6
	overnight	5	5
Proteinase K added during or after lysis (yes/no)?		50 yes, 1 no	50 yes, 2 no
RNase A added during or after lysis (yes/no)?		32 yes, 16 no	32 yes, 16 no

Please indicate below any important details or modifications to the DNA extraction method(s) used.

While T1 DNA extraction from protein concentrate was successful, and we tried it twice (we used ~800ng 1st round and ~500 ng end round), and while measured DNA yield concentrations were OK in both extraction rounds (~20-27 ng/µl measured with Qubit dsDNA kit) we could not get LEC1 to amplify in any qPCR reaction (nor any other event) - we suspect that DNA was highly fragmented. We tested this on 2 separated rounds of qPCR (and analysis done in parallel using same batch of primers and probes worked fine).
Method used: Surefood Prep Advanced by R-Biopharm
Extraction method used for T1 & T2: SureFood® PREP Advanced Kit protocol 1 (r-Biopharm AG)
Purification of 2 - 4 aliquots of lysate per test portion
Both extraction methods are verified in the lab. NucleoSpin Food applied according to manufacturer's instructions, CTAB protocol applied according to Extraction protocol validated for MON863
T1: The volumes of CF lysis buffer and Proteinase K were appropriately upscaled in line with the user manual.
1. Lysis-Step: 45 min, 65°C with RNase A, 2. Lysis-Step: 75 min, 65°C with Proteinase K
For T1 we used more material and larger volume of buffer to bulk replicates of DNA extractions (1 ml of buffer per 100mg material)
Increased sample size (500 mg) and lysis buffer volume (1.5 ml)

What was the average DNA concentration (in ng/uL) obtained (when measured)?

Measurement method*	**DNA concentration**	**T1**	**T2**
By spectrophotometer	<50 ng/µL	6	3
	51-100 ng/µL	6	9
	101-200 ng/µL	9	9
	201-300 ng/µL	4	2
	301-500 ng/µL	3	9
	501->1000 ng/µL	6	4
By Picogreen or similar method	<5 ng/µL	1	1
	6-50 ng/µL	9	1
	51-100 ng/µL	3	4
	101-200 ng/µL	2	5
	>200 ng/µL	0	5

Select the quality control tests performed on the extracted DNA

Answer	**T1**	**T2**
No quality control tests done	2	2
We confirm that the OD ratio @ 260/280 nm is between 1.7 and 2.0	31	30
We confirm that the OD ratio @ 260/230 nm is > 2.0	13	16
We performed a PCR inhibition test on a reference gene target prior to the quantitative analysis (using 2 or more dilutions)	33	34
We performed a PCR inhibition test on a GM gene target prior to the quantitative PCR analysis (using 2 or more dilutions)	5	5
We analysed two or more dilutions of the DNA and compared the qPCR results	18	17
An external positive control was added to the unknown samples	7	7
Other	3	3

If different from above, provide further clarification on the approach used for DNA quality analysis and the outcome

Internal Control present in GMO screen PCR kit
OD ratio 260/230 when using genespin kit is always lower that 2.0
Amplification of an endogenous gene
We confirm that the OD ratio @ 260/280 nm is between 1.6 and 3.0
We analysed more dilutions of the DNA of T1 and compared the dPCR results.
T1, T2: The OD ratio @ 260/280 nm was from 2.09 to 2.15.
Looking at amplification curves
Positive and negative controls were added to the PCR run
GMO screening Kit with IPC

Select the type of PCR tests applied (tick the box when at least one test of the indicated type was applied)

Type of PCR tests	T1	T2
GMO screening	53	52
GM event identification (qualitative)	54	54
GM event quantification	49	48

If screening methods were used (excluding species and event-specific methods), please indicate the results (presence or absence)

Screening target	T1		T2		No Answer
	Present	Absent	Present	Absent	
P35S	12	38	50	1	4
tNOS	12	37	50	1	4
PAT	0	44	0	41	12
BAR	0	26	0	29	25
CP4-EPSPS	0	3	0	3	53
Ctp-CP4-EPSPS	0	0	0	0	56
Ctp2-CP4-EPSPS	2	32	14	28	22
Cry1Ab/Ac	0	21	25	16	34
pFMV	2	21	0	0	29
pNOS	0	2	0	2	54
pNOS-nptII	0	2	0	2	54
t35S	0	1	0	1	55
nptII	0	7	0	7	49
p35S-pat	0	7	0	8	48
p35S-nptII	0	0	0	2	54
pCsVMV-pat	0	4	0	3	52
tE9	0	0	0	11	39
Agrobacterium border seq.	4	1	5	1	50
CaMV	0	2	0	2	54
Other	0	8	1	3	48

Indicate whether the presence of the following GM events (not covered by screening methods) was tested with event-specific methods

GM event	T1		T2		No Answer
	Present	Absent	Present	Absent	
CV127 soybean	0	50	0	0	6
305423 soybean	0	47	0	0	9
40278 maize	0	0	0	48	8
LY038 maize (unauthorised)	0	0	0	20	36
VCO-1981 (unauthorised)	0	0	0	31	25

Further details on other (relevant) screening targets

Soja MON87701, soja MON 87769, soja MON 87751
T2: Multiplex real-time PCR for detection of events without p35S and tNOS (DAS40278, VCO-01981-5, MON87419-8, MON95379): DAS40278, VCO-01981-5, MON87419-8 and MON95379 absent
As the sample had a single event and we found 87708, no other event were further tested. CV127 and 305423 were not tested.
MON95379 was tested negative
T1: p35S/CTP2-CP4-EPSPS/pFMV: Traces, Cq > 37, Agroborder I: absent, Agroborder II: present, P-CsVMV-pat: absent, MON87769 absent, GMB151 absent, MON87708: present↵T2: Agroborder I: absent, Agroborder II: present, P-CsVMV-pat: absent, MON810: absent, GA21: absent, MON87460: absent, MON87430: absent, MON95379: absent, MON89034: present
t35S_pCAMBIA absent in T1 and T2
For T1 we reported "absent" for 40-3-2 soybean as the value obtained did not exceed the method LOD of 0.1% and when quantifying the event a concentration below the method LOQ of 0.08% was obtained.↵For T2 was performed identification and quantification of unauthorised event MON863 (result - 0.3 m/m %).
Also tested in T2: MON863, Bt176, 98140 and DP202216
Absent: Soy: 44406,68416,81419,A2704,A5547 and MON89788; Maize: 1507, 5307, 59122, Bt11,MON87411MON88017, NK603, T25, 98140 and MON863
Screening for 44406, 68416 and 81419 with T-orf23
LY038 not validated in our laboratory

How many independent replicates (separate DNA extractions) were used to obtain the reported quantitative result? 1:T1

Answer	T1	T2
2	24	20

3	6	7
4	12	16
5	4	0
6	2	2
8	1	0

Please report any special observations, e.g. deviations between replicates, between dilutions, etc.

T1: CTAB extracts slightly higher GMO content than NucleoSpin extracts (Av. CTAB 1.58% vs. Nucleo 1.23%) T2 : no observable difference between extraction methods
T1: Measured copy numbers higher than expected with qPCR values. Measured GMO content differs between the extracts within one extraction method. The mean values of the two extraction methods show no obvious differences between the extraction methods. T2: no observable difference between extraction methods
We missed the instruction to mix the powder before taking out for DNA-extraction. Therefor, we obtained for T1 two different GMO contents for each separate DNA extract: Soy MON87708 was T1-1 2.8 % m/m and T1-2 1.7 % m/m. We reported the mean.
The results for T1, which originate from the CTAB extraction, were not taken into account due to the available quality control tests.
Light inhibition in Test item 2
For both extractions rounds, 1 and 2 (each containing A and B samples), we tested 3 template concentrations in duplicate. So for round 1 we had data from 6 technical replicates and for round 2 we had data from 6 technical replicates, so overall data is from 12 technical replicates coming from 4 separate DNA extractions.

Specify the taxon-specific reference target(s) used for quantification, if applicable.

Test item	Reference target	Answers
T1 (Soy protein concentrate)	Le 1 (70 bp) QT-TAX-GM-004	0
	Le 1 (74 bp) QT-TAX-GM-002	31
	Le 1 (74 bp) QT-TAX-GM-005	7
	Le 1 (81 bp) QT-TAX-GM-001	3
	Le 1 (102 bp) QT-TAX-GM-003	1
	Le 1 (102 bp) QT-TAX-GM-020	1
	Le 1 (105 bp) QT-TAX-GM-009	2
	Le 1 (118 bp) QT-TAX-GM-007	2
T2 (maize flakes)	hmg	43
	Adh1 (134/136 bp)	5
	Adh1 (70 bp)	0
	Maize invertase	0
	Other	1

If selected "other", please provide details here

Surefood GMO QUANT MON810 Corn and Surefood GMO QUANT Roundup Ready Soya
QT-TAX-GM-002 and QT-TAX-GM-005 primers and probes are identical, therefore, both targets were marked in the answer to Q5.3

If qPCR was used for GM event quantification, provide the full code of the CRM(s) used for calibration

Test item	GM event	CRM producer	CRM code	Answers
T1	MON87708	AOCS	0311-A	27
			0311-A2	13
T2	MON89034	AOCS	0906-E	29
			0906-E2	13

If you used digital PCR, which general system did you use? Which brand and type of dPCR instrument did you use?

Digital PCR system	Model	Answers
Droplet dPCR, total =8	BioRad QX100	0
	BioRad QX200	8
Chamber dPCR, total =2	Qiagen QIAcuity	2

Did you modify the final concentration of primers and probes when using dPCR for this PT compared to the validated qPCR method? If so, to which concentration and why?

yes: primers and probes according to the manufacturers recommendations (primers: 900 µM; probes. 250 µM)
yes, 500 nM primer each, lab standard protocol for ddPCR
Concentrations of oliognucleotides used for the Lectin-reference gen were doubled to increase signal intensities/resolution
T1: For improved discrimination of the droplet populations the primers were used at a final concentration of 450 nM (taxon) and 600 nM (GM) and probes at a final concentration of250 nM (taxon) and 250 nM (GM). T2: no change to QT-EVE-ZM-018
Final concentrations of primers/probe: 400 nM/200 nM (standardised ddPCR reaction conditions for all assays)
The concentrations of primers and probes for the system LEC was adjusted to 900nM and for MON87708 and MON89034 to 250nM, in order to enhance the resolution. The primer and probe concentrations for HMG are based on the QT-TAX-ZM-007 assay.

Indicate the conversion factor(s) used to convert your dPCR results from a GM copy number ratio to a GM mass fraction.

Test item	GM event	Conversion factor*	Who determined	Answers
T1	MON87708	0.995	Own lab	1
		1.03	JRC	6
		1.04	JRC	2
T2	MON89034	**0.36**	JRC	9
		0.38	Own lab	1
		0.58	JRC	1

* In bold, the recommended CF from the EURL GMFF list version 11 for ERM®-BF412bk (https://gmo-crl.jrc.ec.europa.eu/guidance-documents)

How did you determine the measurement uncertainty associated with your results?

Options	Answers
From the standard deviation of the sample measurements (X2)	15
Using data obtained on routine samples (control charts)	3
Using data from in-house method validation or method verification	17
Following the bottom-up approach described in the GUM (assessing the uncertainties at every step)	2
Using the measurement uncertainty determined by the EURL GMFF from the inter-laboratory trial used for method validation	2
Estimated as a fixed percentage of the result	11
Other	5
Not applicable	1

If selected other, please clarify

According guidance document on measurement of uncertainty for GMO testing lab. 3rd edition
30%
Uncertainty of the conversion factor was also included
u%=2*%RSD/2.83*2.5*xmean/100
According to the Application Note "Use of the harmonised conversion factors to transform PCR results from the DNA copy number ratio domain into the mass fraction domain"
Validation data from kit manufacturer

Based on your measurement results do you consider the sample compliant with the EU GMO legislation, considering that the sample was derived from a product not declared as containing GM material?

See Tables 6 and 7 of this report.

If relevant (e.g. deviating from the normal rules), please clarify your answer.

T1: The quantitative result and the conformity assessment differ from the result of the qPCR. A deviation between the measurement techniques was only observed in the PT material. The positive controls used were measured correctly with both measurement techniques. The measurement results from the dPCR and qPCR do not deviate statistically from each other, but the conformity is determined differently when applying the evaluation criteria.
While we do the analysis, we do not make the final call on compliancy of samples (our monitoring officials make the call on compliancy).
Based on the 'common rounding rules' i.e. the rounding to one number behind the comma, sample T1 is compliant. If we would not apply the common rounding rules, i.e. if we would leave our result as is submitted (1.22 - 0.30 = 0.92 m/m%), we would have classified this result as non compliant to Regulation 1829/2003 (as > 0.90 m/m%)

Additional comments and suggestions

We know that both samples have p35S and T-NOS, so there are typical sequences of genetically modified organisms, but we haven't identified any event in our scope.
Maize DP-915635-4 and DP-Ø23211-2 as approved events were missing as measurands in the report table.
In sample T1, different results appear when analysed with qPCR technique o dPCR technique. In the case of qPCR the sample is not compliant (above 0,9%). In the case of dPCR the sample is compliant (under 0.9%). None of these two methods are already validated in our laboratory for this event.
We observed traces of GTS 40-3-2 below the LOD (0.03 m/m %) in sample T1 (Ct values of 38.7 - 39.8).
We would like to know if/how the T1 sample could be analysed successfully - which extraction method, was the DNA fragment size analysed etc. as we were really surprised when our analysis came out inconclusive (no amplification for Lec1)
The screening results of both samples, T1 and T2, indicate presence of GMOs but we have not been able to identify the specific GMOs present because we do not have the specific technique for those GMOs.

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