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for GM Food & Feed

Determination of GMOs in commercial sheep feed and home-made maize pasta

Report of the EURL GMFF proficiency test GMFF-23/02

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Broothaerts, W., Beaz Hidalgo, M.R., Corbisier, P., Cubría Radío, M., Dehouck, P., Emteborg, H., Robouch, P., Vincent, U.

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Joint Research Centre

Contact information
Name: European Union Reference Laboratory for GM Food and Feed. European Commission, Joint Research Centre
Address: Retieseweg 111, B-2440 Geel - Belgium
Email: JRC-EURL-GMFF-CT@ec.europa.eu

EU Science Hub
https://joint-research-centre.ec.europa.eu

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Abstract

Implementation of the European legislation on genetically modified organisms (GMOs) requires the monitoring of the presence of GMOs in food or feed by analytical tests. 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-23/02" for the determination of GMOs in sheep feed and maize pasta. The two test items analysed in this PT consisted of a commercial sheep feed characterised by the presence of three GM soybean events (T1) and maize pasta spiked with GM maize event 1507 and prepared from maize flour in the laboratory (T2). Fifty-five laboratories participated to the PT round, including 44 National Reference Laboratories (NRLs) from 23 EU Member States, 8 EU Official Control Laboratories (OCLs) and 3 OCLs from EU-neighbouring or EFTA countries, including Switzerland. The evaluation of the analytical performance confirms that most laboratories are able to identify and accurately quantify GMOs in these food and feed samples.

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AGES - Institute for Food Safety Vienna	Austria
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Sciensano	Belgium
Laboratory of SGS Bulgaria	Bulgaria
Croatian Institute of Public Health	Croatia
Croatian Agency for Agriculture and Food, Centre for Seed and Seedlings	Croatia
State General Laboratory	Cyprus
Crop Research Institute	Czech Republic
Danish Veterinary and Food Administration	Denmark
Finnish Customs Laboratory	Finland
Finnish Food Authority	Finland
Service Commun des Laboratoires	France
ANSES	France
AGROLAB LUFA GmbH	Germany
Bavarian Health and Food Safety Authority (LGL)	Germany
CVUA Freiburg	Germany
CVUA-RRW, Krefeld	Germany
Eurofins GeneScan GmbH	Germany
Federal Office of Consumer Protection and Food Safety (BVL)	Germany
Institute for Hygiene and Environment	Germany
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei MV (LALLF MV)	Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt	Germany
Landeslabor Berlin-Brandenburg	Germany
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LAVES	Germany
LTZ Augustenberg	Germany
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Germany
Thüringer Landesamt fuer Verbraucherschutz	Germany
General Chemical State Laboratory (GCSL)	Greece
National Food Chain Safety Institute	Hungary
Eurofins Biomi Kft	Hungary
CREA Centro di Ricerca Difesa e Certificazione	Italy
Istituto Zooprofilattico Sperimentale Lazio e Toscana	Italy
Institute of Food Safety, Animal Health and Environment „BIOR"	Latvia
National Food and Veterinary Risk Assessment Institute NFVRAI	Lithuania
Laboratoire National de Santé	Luxembourg
Wageningen Food Safety Research (WFSR)	Netherlands
Regional Laboratory of Genetically Modified Food	Poland
National Veterinary Research institute	Poland
Plant Breeding and Acclimatization Institute NRI	Poland
INIAV	Portugal
Institute of Diagnosis and Animal Health	Romania
LCCSMS	Romania
A Bio Tech lab ltd	Serbia
SP Laboratorija a.d.	Serbia
Central Control and Testing Institute of Agriculture (CCTIA), Bratislava	Slovakia
State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
National Institute of Biology (NIB)	Slovenia
Centro Nacional de Alimentación (CNA-AESAN OA)	Spain

Organisation	Country
Laboratorio Arbitral Agroalimentario – MAPA	Spain
Laboratorio Central de Veterinaria	Spain
SeLyC	Spain
Swedish Food Agency - Livsmedelsverket	Sweden
Agroscope	Switzerland
Federal Institute of Metrology METAS	Switzerland
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Authors

Wim Broothaerts, European Commission, Joint Research Centre (JRC), Belgium
Roxana Beaz Hidalgo, European Commission, Joint Research Centre (JRC), Belgium
Philippe Corbisier, European Commission, Joint Research Centre (JRC), Belgium
Marta Cubría Radío, European Commission, Joint Research Centre (JRC), Belgium
Pieter Dehouck, European Commission, Joint Research Centre (JRC), Belgium
Håkan Emteborg, European Commission, Joint Research Centre (JRC), Belgium
Piotr Robouch, European Commission, Joint Research Centre (JRC), Belgium
Ursula Vincent, European Commission, Joint Research Centre (JRC), Belgium

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Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised the proficiency test (PT) "GMFF-23/02" for the determination of GMOs in food and feed 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 test items were distributed to participants. Test item T1 consisted of a ground commercial sheep feed containing high concentrations of the soybean GM events 40-3-2, MON87701 and MON89788. Test item T2 was freeze-dried maize pasta prepared in the laboratory from non-GM maize flour and GM maize 1507. The EURL GMFF evaluated the homogeneity and stability of the test items. Because the sheep feed was *labelled as containing GMOs*, the laboratories were requested to identify the GM events and quantify their content in order to assess compliance with the EU GMO legislation. While no assigned values were determined for the soybean GM events in this test material, the focus was on evaluating the approaches used by the laboratories to verify GMO claims on a product. The assigned value for T2 (*not labelled as containing GMOs*) was derived from the results reported by four expert laboratories.

Fifty-five laboratories participated to the PT round, comprising 44 NRLs from 23 EU Member States, 8 EU OCLs, and 3 OCLs from EU-neighbouring or EFTA countries. Two laboratories indicated that T1 (feed) fell outside their laboratory's scope and was not analysed, reducing the total number of reporting laboratories to 53 for T1.

The first step in GMO analysis, following DNA extraction, is the qualitative identification of any GM event(s) present in the test 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 potentially remaining GM events. Over 91 % of the laboratories identified correctly the soybean events (40-3-2, MON87701 and MON89788) in T1, and the 1507 event in T2.

Quantification of the GM soybean events in T1 was needed to assess compliance of the product. The vast majority (above 85 %) of the laboratories reported quantitative results for the identified GM soybean events. As the GM contents exceeded the labelling threshold significantly, many laboratories reported values as "larger than", consistent with the validated dynamic range of their methods. Most of the reported results were in line with the indicative GM contents presented in this report.

The quantitative results reported for the 1507 event in T2 were evaluated using z prime (z') and zeta (ζ) scores, in accordance with ISO 13528:2022 [3]. The relative standard deviation for proficiency assessment (σ_{pt}) for this GM event was set to 25 % of the assigned value, based on the experience acquired from previous PT rounds.

Forty-eight of the 55 laboratories reported a quantitative result for 1507 in T2, and 5 of them provided results obtained by qPCR and dPCR. The vast majority of the results (94 %) were considered satisfactory, as expressed by their z' scores, whereas the remaining three results were flagged as questionable. The variation between the dPCR results appeared much smaller and closer to the assigned value in comparison to the qPCR results reported by the same laboratories. Most participants reported a realistic expanded measurement uncertainty and coverage factor for their results.

Participants were also asked to evaluate the compliance of the test items against the applicable EU legislation on GMOs. In total, 88 % of the laboratories that assessed the compliance of both test items issued accurate compliance statements, confirming that T1 was correctly labelled as containing GMOs above the labelling threshold in Regulation (EC) No 1829/2003, while T2 should have been labelled in accordance with the same Regulation.

This PT round confirms that most NRLs and OCLs are able to monitor and quantify the mass fractions of GMOs in food and feed samples within the scope of Regulation (EU) 2017/625.

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 of three GM soybean events in sheep feed and the determination of the mass fraction of GM maize event 1507 in maize pasta, 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, 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 test items were prepared and dispatched to participants for analysis. Sheep feed (test item T1) was selected to resemble a commercial feed product regularly analysed by control laboratories in the EU. The second sample (food, test item T2) consisted of ground maize pasta prepared from commercial maize flour and ground seeds of GM maize event 1507.

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 and feed products and the assessment of the compliance of the samples in relation to the applicable EU Regulations (EC) No 1829/2003 [5] and (EU) No 619/2011 [6].

The PT was mandatory for the 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-23/02".

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	28 August 2023
Registration deadline	10 September 2023
Sample dispatch	26 September 2023
Results deadline	10 November 2023

3.4 Distribution

Each participant received:

- One bottle of test item T1 (ground sheep feed), containing approx. 5 g of dry powder;
- One bottle of test item T2 (ground maize pasta), containing approx. 5 g of dry powder;
- A general "Test item accompanying letter" (Annex 2).

Samples were dispatched at room temperature. Participants were asked to check whether the bottles were damaged after transport and to store the test items in a cool place at approximately 4 °C.

3.5 Instructions to participants

Detailed instructions were given to 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 test items were described as follows:

- T1 is a ground feed test material "*declared as containing GM material"*.
- T2 is a ground food test material "*derived from a product that is not declared as containing GM material*".

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

Test Item 1, "*containing GM material"*:

- To identify all GM soybean events in this sample;
- To quantify the GM content in line with their routine procedures, for assessing the compliance of the sample with the applicable GMO legislation.

Participants were informed that while the analysis results reported for T1 would be assessed for GM event identification, no performance scores would be calculated for the quantitative results submitted. Instead, the quantitative results would only be used to evaluate the accuracy of the compliance statement provided.

Test Item 2, "*derived from a product that is not declared as containing GM material*"

- To verify the presence of GM maize in this sample;
- To quantify the GM event identified and assess compliance of the sample with the applicable GMO legislation.

Participants were informed that the procedure used for the analysis should resemble as closely as possible their routine procedures for this type of matrix and GM mass fraction levels. The quantitative results had to be expressed in "mass/mass %". As the homogeneity study was conducted using a 200 mg sample intake for both T1 and T2, this amount was set as the recommended minimum sample intake.

When submitting their results, participants were instructed (i) to select the appropriate option (e.g. "not tested", "present", "absent" 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 reporting 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 to collect additional information related to the methods used by the laboratories when performing the measurements.

4 Test items

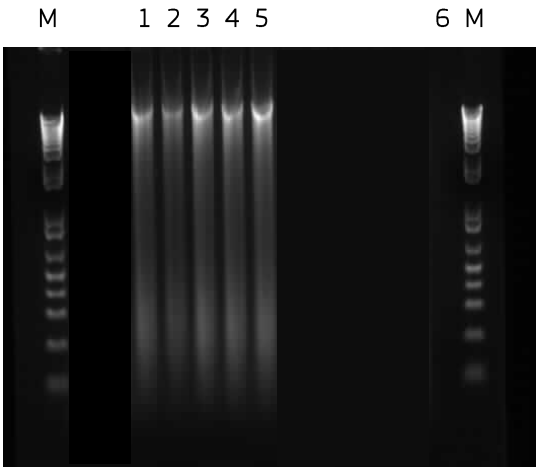
4.1 Preparation

Test item T1 consisted of a feed material for sheep, previously cryo-ground for use as a test material by the EURL for Feed Additives in 2019. An aliquot was tested for the presence of GMOs and a high content of three GM soybean events was detected. The material was used in its original form without further spiking with other GM materials.

To reduce the water content (following dry-mixing in a Dyna-Mix 200), the material was vacuum-dried and dry-mixed again. The final water content was 1.49 ± 0.22 g/100 g (mean ± *U, k*=2; *n*=3). The T1 mixture was manually filled using a vibrating feeder and a balance into 20 mL glass vials (ca. 5 g per vial) and closed under argon. 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 125 vials were produced.

The amount and the quality of the DNA extracted from the T1 material using a CTAB method with Genomic-tip 20 purification were verified by UV spectrometry, fluorometry, and gel electrophoresis (Figure 1). The DNA appeared as a smear on the gel, indicating partial degradation. A selection of DNA extracts was tested for PCR inhibition with the soybean reference gene *Lec* target (74 bp) using four serial dilutions and passed the evaluation criteria (slope and ΔCq). A qualitative analysis for GM events using screening and event-specific pre-spotted plates [7, 8] indicated the presence of three soybean events at low Cq values (40-3-2, MON87701, MON89788) and traces of A5547 soybean and several maize events (T25, 59122, MIR162, MON863 and MON89034).

Figure 1. Agarose (1 %) gel electrophoresis of genomic DNA extracted from the T1 material (lanes 1-5), lane 6 is an extraction blank. The molecular marker in the first and last lane (M) is the 1 kb Plus DNA ladder (Invitrogen, USA). Other lanes on the gel image have been blacked.



Test item T2 consisted of ground maize pasta dough prepared in the laboratory from non-GM maize flour and ground 1507 maize seeds (previously used for the production of the CRM ERM-BF418), with addition of olive oil and sea salt (no eggs). The dough was spread into a layer of approximately 0.5 cm, then freeze-dried, milled and filled in 5 g portions into 20 mL vials, closed under argon. A total of 125 vials were produced. Further details on the processing can be found in Table 1.

The amount and the quality of the DNA extracted from the T2 material using a CTAB extraction method (without Genomic-tip 20 purification) were verified by UV spectrometry, fluorometry and gel electrophoresis (Figure 2). The DNA appears as a smear on the gel, indicating partial degradation. A selection of DNA extracts were tested for inhibition with the maize reference gene *hmg* target using four serial dilutions and passed the evaluation criteria (slope and ΔCq).

Figure 2. Agarose (1 %) gel electrophoresis of genomic DNA extracted from the T2 material (lanes 1-6). The molecular marker in the first lane (M) is the 1 kb Plus DNA ladder (Invitrogen, USA).

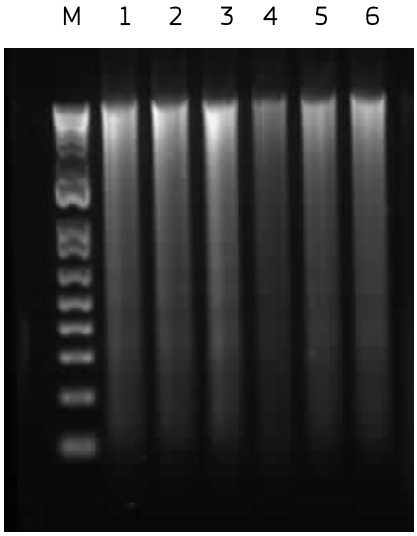


Table 1. Characteristics of the base materials used for the preparation of T2

Characteristic	Non-GM Soybean	GM maize 1507
Type of base material	100 % maize meal	Ground seeds of 1507 maize
Origin	'Maismeel' from Dipro, from biological agriculture	Left-over from production of ERM-BF418
Mass used to prepare T2 (g) – STEP 1	100.05	11.25
Mass used to prepare T2 (g) – STEP 2	638.78	111.21 g of step 1
Mixing equipment	DynaMIX CM-200	
Processing	Addition of water, olive oil and sea salt, kneading by hand in the laboratory, spreading on flat plate	
Freeze-drying	Martin Christ FD 1-6D	
Grinding	Vibrating cryogenic mill Palla VM-KT	
Sieving	Russel sieve 710 µm	
Milling	DynaMIX CM-200	
Water content in g/100 g, mean ± *U* (*k*=2), with *n*=3	5.52 ± 0.82	
Particle diameter in µm, mean ± *U*[1] (*k*=2), with *n*=3	29.54 ± 0.98	

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

4.2 Homogeneity and stability

Measurements for the homogeneity and stability studies were carried out by the EURL GMFF using the corresponding validated event-specific detection methods, with *le1* (74 bp) and *hmg* (79 bp) as taxon-specific reference target for T1 and T2, respectively. The homogeneity of T1 was evaluated by monitoring only the 40-3-2 event. The statistical treatment of the data was also performed by the EURL GMFF.

The assessment of **homogeneity** was performed after the processing and bottling of the test items. Ten bottles (of T1 and T2) were randomly selected and 3 independent replicates per bottle were used for homogeneity analysis. DNA extraction was done with CTAB+genomic-tip 20 for T1 and CTAB (without tip 20) for T2. qPCR results were evaluated according to ISO 13528:2022 [3]. The contribution from homogeneity (u_{hom}) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated according to ISO Guide 35:2017. The T1 and T2 materials proved to be adequately homogeneous for the two GM events investigated (Annex 4.1).

The stability under dispatch conditions was assessed for T1 and T2. It was performed using an isochronous short-term stability scheme [9] involving two test samples with three replicates each (N=2, n=3) and conducted over one week at +20 °C or +40 °C. The measurements by qPCR were performed under repeatability conditions. The results revealed no significant influence of storage at +20 °C or +40 °C on the stability of either test item compared to storage at a reference temperature of -18 °C (data not shown). The materials were therefore dispatched at room temperature.

The **long-term stability** during the extended period covered by the PT round was tested using qPCR, analysing the GM content in bottles (N=2, n=3) stored at the normal storage temperature of +4 °C. This was done for T2 only, as no assigned values were determined for T1 and no performance scoring was done for this test item. The normal storage temperature of +4 °C has been shown to be fit for the purpose of ensuring stability in similar samples used in previous studies. Participants were also instructed to store the samples at +4 °C upon reception and until analysis.

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 (i.e. increase or decrease due to storage). No significant trend was detected at a 95 % confidence level (Annex 4.2). This stability study confirmed that both test items remained adequately stable at +4 °C during the whole time period of the PT round. Hence, the uncertainty contribution to the assigned value due to instability was set to zero (u_{stab}=0) [3].

5 Assigned values and corresponding uncertainties

5.1 Assigned values

Assigned values were not calculated for T1 due to the presence of three GM soybean events at contents significantly exceeding both the labelling threshold of 0.9 m/m % and the validated dynamic range of the methods used by the laboratories. The participants were consequently requested to determine the GM content at a suitable (semi-quantitative or quantitative) level to assess sample compliance. However, indicative content values for the three GM soybean events in this feed material are provided in Table 2. These values were derived from measurements conducted by the EURL GMFF (Geel) laboratory and, specifically for 40-3-2 event, by three additional external expert laboratories.

Table 2. Indicative GM soybean content values in T1.

Test item	**GM soybean events**	**indicative content** in m/m %
T1 (sheep feed)	40-3-2	Approx. 39
	MON87701	Approx. 37
	MON89788	Approx. 30

The assigned value (x_{pt}) for the mass fraction of the 1507 event in T2 was derived from measurement results obtained by qPCR in the EURL GMFF (Geel) and three external laboratories, selected based on their performance in previous EURL GMFF PT rounds (2018-2022) and using the appropriate accredited methods. Each external laboratory was free to apply a DNA extraction method of its choice, provided that the DNA quality ($OD_{260/280}$ and $OD_{260/230}$ ratios and PCR inhibition test for an endogene target) and quantity were acceptable for qPCR.

The expert laboratories analysed two bottles of each test item, performed five independent DNA extractions from each bottle, and reported 10 results for each test item.

The assigned value was calculated as the mean of the average results reported by the four expert laboratories (Table 3), in line with ISO 13528:2022 - Section 7.6 [3].

This value is almost three times larger than the nominal fraction of 1.5 % of 1507 maize in T1 (derived from the gravimetric preparation). This may be attributed to a lower DNA extractability of the non-GM maize meal used, combined with processing of T2. This discrepancy between the nominal value and the measured value was, however, not further investigated.

Table 3. Assigned value (x_{pt}) and standard deviation for the proficiency assessment (σ_{pt}) for the 1507 event in T2

Laboratory	**DNA extraction method**	**Average ± *U* (*k*=2)**	x_{pt} m/m %	u_{char} m/m %	u_{hom} m/m %	$u(x_{pt})$, *k=1* m/m %	σ_{pt} m/m %	$u(x_{pt})/\sigma_{pt}$
Lab 1	CTAB	4.30 ± 1.78	**4.38**	0.37 (8.4 %)	0.14 (3.2 %)	**0.39** (9.0 %)	1.16 (25 %)	0.36
Lab 2	CTAB+Maxwell automated extractor	5.39 ± 2.62						
Lab 3	NucleoSpin Food	4.21 ± 0.30						
Lab 4	NucleoSpin Food	3.63 ± 0.76						

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 "*s*" refers to the standard deviation of the "*p*" dataset means and "*p*" refers to the number of datasets.

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;
- all the values reported by the expert laboratories were traceable to the SI unit through 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 (Table 3).

6 Scores and evaluation criteria

Laboratory competence in qualitatively identifying a GM event in a test 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 electronic 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 test items.

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]:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u^2(x_{pt})}} \qquad \text{Eq. 3}$$

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

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.

Note: The z' score was applied, following the recommendation outlined in the ISO 13528:2022 standard [3], due to the uncertainty of the assigned value $u(x_{pt})$ exceeding 0.3 σ_{pt} (See Table 3, event 1507).

The interpretation of the *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' 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 [10].

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 test 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.

It should be understood that the reported data from participants were not $\log_{10}$-transformed prior to the performance assessment [11].

7 Evaluation of reported results

7.1 Participants

Forty-five NRLs and eleven OCLs initially registered to this PT round. However, one NRL later declined participation (Table 4), resulting in a total of 55 participants. NRLs responsible for managing official controls under Regulation (EU) 2017/625 (NRL/625) constituted 56 % of all participants. All EU Member States (except Malta) contributed to this PT round. According to our knowledge, Estonia and Ireland designated AGES in Austria and Wageningen Food Safety Research (WFSR) in The Netherlands, respectively, as their NRL for GMO analysis. Furthermore, three additional NRL/625 laboratories agreed to conduct characterisation measurements for this PT, identified as 'certifiers' in Table 4. Consequently, they were not considered as 'regular' participants to this PT.

Table 4. Overview of participants to GMFF-23/02 by country and category

Country	**Participants**	**NRL/625**	**NRL/120 (and not NRL/625)**	**OCL (not NRL)**
Austria	2	2		
Belgium	3	3		
Bulgaria	1	1		
Croatia	2	2		
Cyprus	1	1		
Czech Republic	1	1		
Denmark	1	1		
Estonia (represented by AGES, AT)				
Finland	2	1	1	
France	2	2		
Germany	15	1	11	3
Greece	1	1		
Hungary	2	1		1
Ireland (represented by WFSR, NL)				
Italy	1	(1 certifier)	1	
Latvia	1	1		
Lithuania	1	1		
Luxembourg	1	1		
Malta	0			
Netherlands	0	1		
Poland	3	3		
Portugal	1	1		
Romania	2	1		1
Serbia	2			2
Slovakia	1	1 (+ 1 certifier)		
Slovenia	0	(1 certifier)		
Spain	4	3		1
Sweden	1	1		
Switzerland	2			2
Turkey	1			1
Total (excluding certifiers)	**55**	**31**	**13**	**11**

7.2 Qualitative results

The first task requested from the participants was to identify the soybean or maize GM event(s) present in the test 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.

In T1, the following screening elements could have been positively scored:

- From 40-3-2: P35S, T-nos, CTP-CP4-EPSPS;
- From MON87701: Cry1Ab/Ac, Cry1Ab;
- From MON89788: CTP2-CP4-EPSPS, pFMV, tE9.

Additionally, PAT (and P35S – PAT) could potentially have been detected as weakly positive due to traces of A5547 soybean. Furthermore, traces of several maize events might have produced weakly positive signals, although it is uncertain if these events would have been consistently detected.

In T2, the following screening elements could have been positively scored, while all other elements (and constructs) should have been scored as "absent":

- From 1507: p35S, PAT, P35S-PAT, PAT-T35S.

The screening results reported by the laboratories are shown in Annex 5. Positive detection of the elements related to the main GM events indicated above is denoted in green, while the absence of these elements is indicated in red, as they should have been detected if the corresponding screening method was applied. Due to the presence of trace levels of additional GM events in T1, the results of (P35S-)PAT, (P35S-)nptII, Agro and CMV screening tests were not scored as correct or not in this test item. For T2, where traces of MON810 were reported by some laboratories, the presence or absence of Cry1Ab/Ac was not evaluated as correct or incorrect.

The evaluation of the screening results showed that a large majority of the laboratories had performed the screening tests correctly. Only a few laboratories had reported false positive or false negative results for some of the tests applied. Particularly, it is recommended that laboratory L09 review the parameters of its screening tests or their evaluation process.

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

In **T1**, more than 93 % of the laboratories successfully identified the soybean events 40-3-2, MON87701 and MON89788. However, three laboratories reported 'absent' for one or two of these events, failing therefore to detect them. For L45, the non-detection of MON87701 appears to be linked to the non-detection of the Cry1Ab/Ac element during screening (Annex 6). Despite the request to identify all GM events, two NRL/625 laboratories (as well as two OCLs) did not test for some of these events.

For **T2**, 91 % of the participants successfully identified the 1507 event. Four laboratories did not test for this event, and L22 did not detect it - they reported 'absent' for 1507, but MON810 as 'present' at <0.1 m/m %. Event MON810 was also identified by a number of other laboratories, predominantly reporting 'less than' values (< 0.1 m/m %), although some laboratories reported quantitative values (e.g. 0.01 %; 0.02 %; or 0.1 %). While we cannot confirm with analytical evidence that traces of MON810 are present in T2, it is plausible that the 1507 seed powder used for preparation of this test item may have been contaminated with this event.

In conclusion, a significant majority of the laboratories that tested the sample and corresponding GM event(s) demonstrated their capability to identify the correct GM event(s) in the animal feed and food matrix. However, it remains unclear whether the laboratories that indicated "absent" in the reporting form truly failed to detect the event or inadvertently selected the term "absent" from the drop-down list.

Table 5. Summary of the reported qualitative identification of the GM events in T1 and T2, expressed as number of laboratories (green=correct, red=wrong, orange=not tested)

Sample analysed?	Outcome	Test item T1			T2
		40-3-2	MON87701	MON89788	1507
Analysed	Detected	51	49	48	50
	Not detected	1 (L06)	2 (L45, L50)	1 (L50)	1 (L22)*
	Not tested	1 (L09)	2 (L19, L28)	4 (L09, L19, L28, L37)	4 (L09, L11, L23, L37)
Not analysed		2 (L12, L58)			-

* L22 only reported MON810 as < 0.1 m/m %

7.3 Quantitative results

7.3.1 Performance

The number and type of quantitative results reported for T1 and T2 are shown in Table 6.

As previously mentioned and indicated in the instructions letter for this PT, performance scores were not calculated for the reported results for T1. The reason for this is that the GM content of each of the GM events in this commercial feed sample significantly exceeds the legal threshold for labelling and may fell outside the validated dynamic range of the GMO detection methods. Consequently, measurements at this level may not be reliable. The reported results are, therefore, presented in Annex 6 with no performance scores.

The questionnaire collected additional details on the validated dynamic range for the soybean GMO methods used for T1. Approximately half of the laboratories conducted in-house validated of the three detection methods for GM soybean between 0.1 (or lower) and 10 m/m % GM, in line with the dynamic range specified (0.1 to 9 or 10 m/m %) in the validation report of each of these methods in the GMO METHODS database of the EURL GMFF. The remaining laboratories extended their range to include values close to 100 % GM, with at least three laboratories re-validating this extended range to include higher GM % specifically for this PT. When asked about the protocol for samples with GM percentages exceeding the validated dynamic range, thirteen laboratories indicated that they dilute the sample to bring measurements within the range of the method. In contrast, 24 laboratories stated that they would report the result as “larger than the upper level of the dynamic range”, although some of them provided an actual value in this PT. In response to the questionnaire, several laboratories agreed that measuring such high GM% is not relevant in view of the legislative requirements.

Table 6. Summary of the quantitative results reported for the GM events in T1 and T2, expressed as number of laboratories

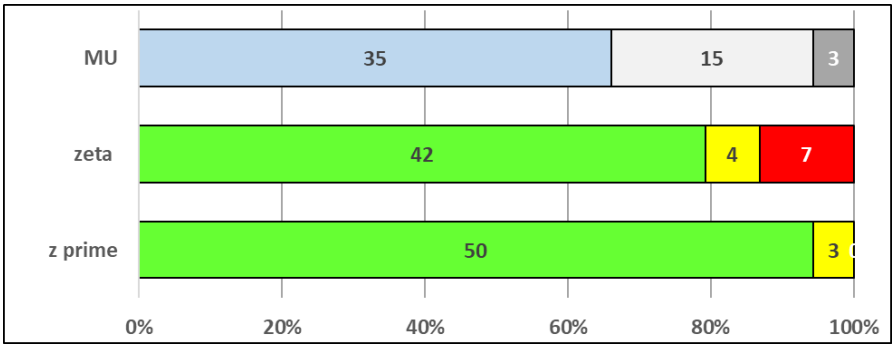
Reported quantitative result	PCR system	T1			T2
		40-3-2	MON87701	MON89788	1507
Absolute value	qPCR	32	31	31	43
	qPCR and dPCR	3	2	2	5
Truncated value	qPCR	14	12	12	-
No value		4	8	8	7
Total number of laboratories		53	53	53	55

Instead of performance scoring for T1, the correct assessment of sample compliance, in line with the reported results, was evaluated (see Section 7.4 below). For this assessment, the presence of truncated values 'larger than' a value above the labelling threshold is perfectly acceptable. Several laboratories have reported truncated values, assumingly following their routine procedure for reporting results for samples with such high GM content.

For T2, laboratory performance in quantifying the GM event (1507 maize) was assessed using *z'* and zeta (ζ) scores. Figure 3 summarises the laboratories' performance, while Annex 6 presents the reported results for the 1507 maize measurand. 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 [12].

Performance scores were assigned to 53 results reported by 48 laboratories, with 5 laboratories reported results obtained by both qPCR and dPCR, as indicated in Table 6. The vast majority of the results (94 %) were considered satisfactory, as expressed by their z' scores, whereas the remaining three results were flagged as questionable. The seven unsatisfactory zeta (ζ) scores obtained were due to either an underestimation or overestimation of the GM content, often in conjunction with an underestimated measurement uncertainty.

Figure 3. Overview of laboratory performance according to *z'* and ζ scores, for the content of the event 1507 maize in T2.



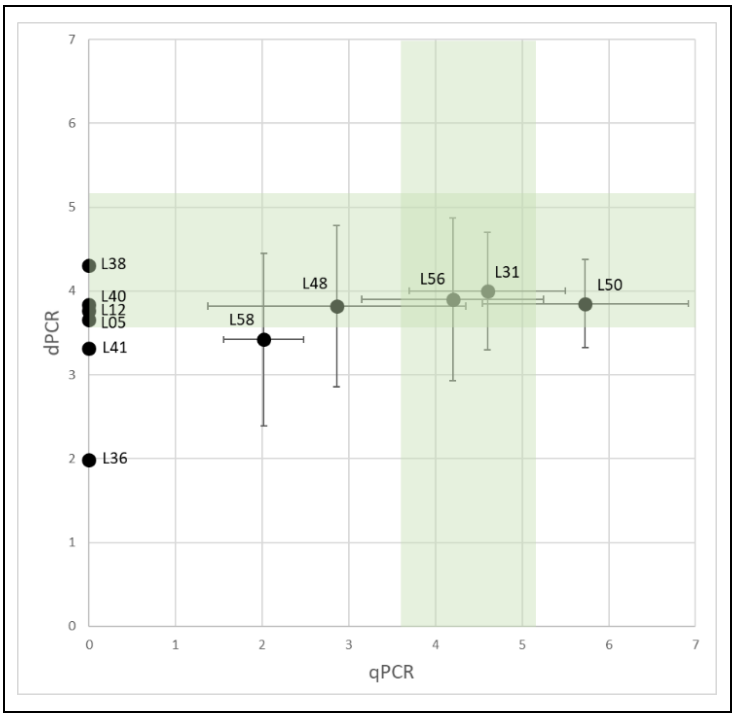
Satisfactory, questionable and unsatisfactory performance scores are indicated in green, yellow and red, respectively. Corresponding numbers of results are shown in the bars. Measurement uncertainty (MU) was evaluated as follows: Case "a" (blue): $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$;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

7.3.2 Digital PCR results

Eleven laboratories reported **digital PCR** results for 1507 maize in T2, with five of them reporting both qPCR and dPCR results. Among these, the dPCR results from the five laboratories were notably closer to the assigned range compared to their corresponding qPCR results (Figure 4). Additionally, five out of the remaining six dPCR results were also close to this range. Similar results were observed between a chamber dPCR instrument (QIAcuity; L48 and L50) and droplet dPCR (BioRad).

Most laboratories applied a conversion factor of 0.61 to convert their dPCR results (expressed in copy number ratio) into a corresponding GM mass fraction for 1507 maize, in line with the recommendation by the EURL GMFF (Guidance documents | European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) (europa.eu)). However, L05 (OCL) and L41 (NRL/120) applied slightly different conversion factors of 0.637 and 0.659, respectively, which is not in line with the EURL GMFF recommendation.

Figure 4. qPCR versus dPCR results (in m/m %) reported for 1507 maize in T2. The green areas represent the assigned range ($x_{pt} \pm U$). dPCR results for which no corresponding qPCR result was reported are shown on the Y axis (without measurement uncertainty).



7.3.3 Truncated values

Approximately 12 to 14 laboratories reported truncated (larger than) values for the soybean GM events in T1, often exceeding 5 or 10 m/m %. These values are considered plausible and were included as such in the evaluation of the compliance assessment.

7.3.4 Measurement uncertainties

All laboratories that reported quantitative results, with the exception of L06 for 1507 maize (OCL), provided an expanded measurement uncertainty and a coverage factor (Annex 6). The majority of these laboratories reported a realistic measurement uncertainty (Case "a" in Figure 3).

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 (for authorised GMOs in food and feed, labelling if > 0.9 m/m %) or Regulation (EU) No 619/2011 (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 7-8. Some laboratories provided a justification for their selected compliance options, while others did not reply to the questions on compliance. 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. L58 indicated in the questionnaire that only their Competent Authority is authorised to assess compliance of a tested product.

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 the report the (highest) result (x_i) and its expanded measurement uncertainty (U), calculate the "xi – U" value, and finally evaluate whether the resulting value exceeded the threshold specified in the applicable Regulation.

The GM soybean events present in **T1** are authorised in the EU, therefore the reported range (result ± expanded uncertainty) is to be compared to the labelling threshold of 0.9 % (m/m), as stipulated by Regulation (EC) No 1829/2003 (assuming no GM events were identified that were not or not yet authorised in the EU). The following assumptions were taken into account:

- The product information provided indicated that the sheep feed was labelled as containing GMOs, as indicated in the instructions letter.
- The content of each of the three soybean events measured in T1 was significantly above the threshold, indicating that labelling of the product is clearly required. Therefore, the product, labelled as containing GMOs, is deemed compliant.

The large majority (89 %) of the laboratories that issued a compliance statement in the questionnaire (42 out of 47) selected the correct option, indicating that the T1 product, labelled as containing GMOs, complies with the labelling rules under Regulation (EC) No 1829/2003 (Table 7). However, three laboratories declared that it was not compliant, despite their results confirming the GM content to be significantly above 0.9 m/m % - they probably did not consider the fact that the product was labelled as containing GMOs. Additionally, L38 reported a high GM content, but indicated that the sample complied with Regulation (EU) No 619/2011, even though no GM events were identified that are listed in the EU GM register with pending or expired authorisation status.

Table 7. Reported compliance statements for T1 (sheep feed) based on the number of laboratories

Compliance Statement	Laboratory Measurement	Number of Laboratories	Comment
Compliant, because product was labelled	$x \pm U \leq 0.9$ m/m %	0	
	$x \pm U > 0.9$ m/m %	42	
Product should not have been labelled (not compliant)	$x \pm U > 0.9$ m/m %	3	L11, L16, L27
	$x \pm U \leq 0.9$ m/m %	0	
C<LLP - Compliant, under Regulation 619/2011 but ≤0.1 m/m %, in feed	$x \pm U \leq 0.1$ m/m %	1	L38; wrong as this Regulation does not apply
NC>LLP - Not compliant, under Regulation 619/2011 and >0.1 m/m %, in feed	$x \pm U > 0.1$ m/m %	2	L11, L32; wrong as this Regulation does not apply
CNC - Cannot conclude / not quantified		1	
Total number of laboratories with a compliance statement[1]		48	

[1] L11 has selected more than one option

For **T2,** a similar assessment was made. The 1507 maize event is authorised in the EU, therefore the reported range (result ± expanded uncertainty) is to be compared to the labelling threshold of 0.9 m/m %, with only this Regulation applying. The following assumptions were considered:

- The measured content of 1507 in T2 (4.38 m/m %) exceeds the threshold.
- The lower limit of the assigned (expanded, with coverage factor 2) range for 1507 is 4.381 - 0.788 = 3.593 m/m %. Considering the number of significant figures in the legislation, the measured value needs to be rounded to 3.6 m/m %. Hence, labelling is required for this material. Since the product is not labelled as GMO (as indicated in the instructions letter for this PT), it is deemed non-compliant.

All laboratories, except one (44 out of 45, i.e. 98 %), selected the correct compliance statement (Table 8).

Table 8. Reported compliance statements for T2 (maize pasta) based on the number of laboratories

Compliance Statement	Laboratory Measurement	Number of Laboratories [a]	Comment
CNL - Compliant, because no labelling required	$x \pm U \leq 0.9$ m/m %	0	
	$x \pm U > 0.9$ m/m %	1	L45
NCL - Not compliant, should have been labelled	$x \pm U > 0.9$ m/m %	44	
	$x \pm U \leq 0.9$ m/m %	0	
C<LLP - Compliant, under Regulation 619/2011 but ≤0.1 m/m %, in feed	$x \pm U \leq 0.1$ m/m %	0	
NC>LLP - Not compliant, under Regulation 619/2011 and >0.1 m/m %, in feed	$x \pm U > 0.1$ m/m %	0	
CNC - Cannot conclude / not quantified		5	
Total no. of compliance statements		50	

7.5 Questionnaire

The questionnaire was answered by all but two participants (L21 and L51). As four participants provided separate answers for their qPCR and dPCR results the total number of answers received was 57 (from 53 laboratories). The results provide valuable information about the participating laboratories, their analysis strategy and analytical approaches. 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.

All laboratories, except L12 and L58 (who tested only T2), analysed both test items. The majority of participants reported that their laboratory was accredited in accordance with **ISO/IEC 17025** for the DNA extraction method employed (33 with a flexible, 14 with a fixed scope) and the qualitative screening methods (34 flexible, 12 fixed scope) used in the PT round. Fewer laboratories were accredited for the quantitative event-specific methods, i.e. for T1 and T2, respectively, 7 and 6 laboratories with a fixed scope and 29 and 26 with a flexible scope. In addition, 9 (T1) and 4 (T2) laboratories responded to have only some of the applied quantitative event-specific methods under accreditation. A few laboratories obtained accreditation for the dPCR method(s) used for T1 and T2, including 3 with a fixed scope, 5 with a flexible scope, one with a mixed (fixed and flexible) scope, and one with some of the dPCR methods covered.

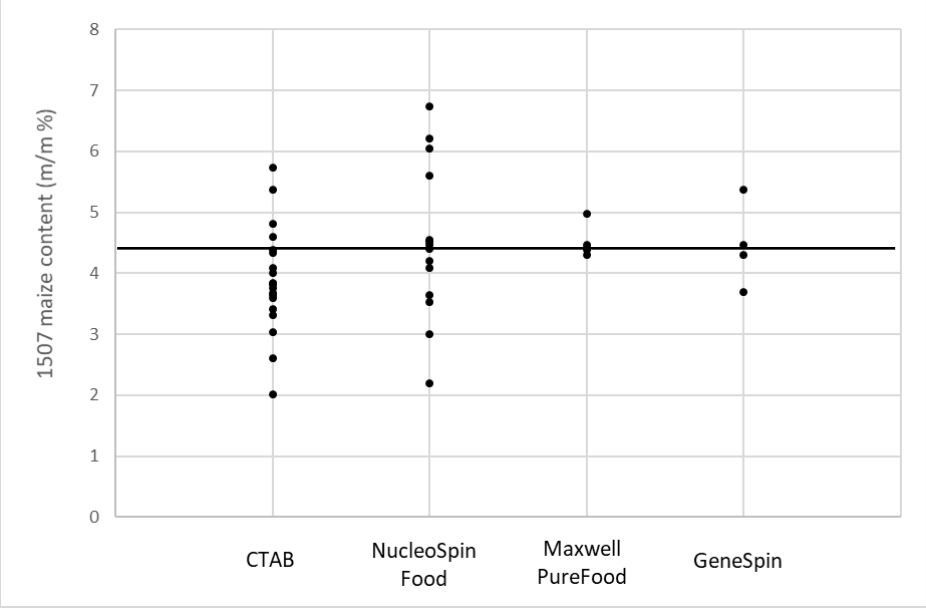
The outcome of the **screening methods** used for T1 and/or T2 were summarised (Annex 5) and discussed before (Section 7.2).

The questionnaire also includes a question on the type of digital PCR instrument used, as well as whether modifications were made to primer and probe concentration for dPCR compared to the corresponding validated qPCR method. It was noted that many laboratories modified primer concentrations to a range between 400-900 nM and probe concentrations to 100-250 nM to achieve optimal resolution. The conversion factor applied was in most cases the one recommended by the EURL GMFF, although some laboratories continue using the not-recommended and slightly deviating conversion factor determined in their laboratory.

Several questions addressed the dynamic range of the applied methods and the laboratory's strategy in cases where a sample contains a GM % outside this range (as discussed in section 7.3.1).

Of particular interest is to verify whether **the DNA extraction method employed impacted the reported GM content** (for T2). Different methods were used by the laboratories, mostly based on the use of (1 or 2 %) CTAB for lysis (20 results), or using commercial kits such as NucleoSpin Food (14), Maxwell RSC PureFood (5), or GeneSpin (4). Comparison of the reported results did not reveal an effect of the DNA extraction method used on the results obtained in the laboratories for T2, based on the most frequently used extraction methods (Figure 5). However, the highest variation in the reported results is observed after NucleoSpin Food extraction, as well as after use of one of the CTAB protocols.

Figure 5. Effect of DNA extraction method used on reported GM quantity for T2. Horizontal line: assigned value.



8 Conclusions

The proficiency test GMFF-23/02 was organised to assess the analytical capabilities of EU NRLs and OCLs to analyse a feed material (T1) and a food material (T2), with the objective of identifying the GM events present and determining the GM content in these test items.

The vast majority of participants correctly identified the three GM soybean events in T1, with most of these laboratories quantifying these GM events to assess product compliance. Given that the GM content in T1 exceeded the legal labelling threshold by a significant margin, it triggered discussions regarding whether measurements at such high GM content levels fall within the dynamic range of the method.

Nearly all laboratories identified the 1507 maize event in T2 and quantified its content. The analytical performance of the participants in determining the 1507 content in T1 was mostly satisfactory (94 %). The reported dPCR results were generally closer to the assigned value compared to the qPCR results.

The compliance statements provided by the majority of laboratories were considered consistent with their reported results for T1 and T2. This demonstrates that control laboratories are generally competent to assess food and feed products on the EU market for the presence of GMOs and confirms their analytical capabilities to enforce the EU GMO regulations [13].

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[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). Available from 32011R0619.

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List of abbreviations and symbols

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
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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Annex 1. Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 28 August 2023
JRC.F.5/UV/wb/mt/ARES(2023) 23-060

FOR THE ATTENTION OF THE
NATIONAL REFERENCE LABORATORIES (NRLS) FOR GMOS
UNDER REGULATION (EU) 2017/625 (AND SELECTED NON-EU OFFICIAL LABORATORIES)

Subject: Invitation to participate to the Proficiency Testing round "GMFF-23/02"

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 sheep feed (T1) and GM maize in homemade milled maize pasta (T2)**". You will receive two ground test materials.

- For T1, labelled as GM, you are requested to check for the presence of all GM soybean events (identification), and quantify them, in line with your routine procedures, for assessing the compliance of the sample with the applicable GMO legislation.
- For T2, not labelled as containing GMOs, you are requested to check for the presence of GM maize, quantify the identified GM event, and assess the compliance of the sample 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.
https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=2941

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.**

Please register by Sunday 10 September 2023.

The test items will be shipped on Tuesday 26 September 2023.

The deadline for submission of the results is Sunday 12 November 2023.

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.

/signed electronically in Ares/

Dr. Ursula Vincent
Head of Unit

/signed electronically in Ares/

Dr. Wim Broothaerts
PT Coordinator

Annex 2. Test item accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance

European Union Reference Laboratory for GM Food & Feed

Geel, 26 September 2023

Subject: **GMFF-23/02, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* sheep feed and milled maize pasta**

Dear participant,

Thank you for participating to this PT round. Please find in this parcel two test materials, T1 and T2, each consisting of 5 g of ground sample.

Upon arrival, you should immediately store the samples in a fridge at ~4 °C.

Please check whether the bottles remained undamaged during transport and inform us if they arrived later than one week from the date of this letter. We will promptly replace any damaged test items.

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 (Geel)
Food and Feed Compliance

European Union Reference Laboratory for GM Food & Feed

Geel, 26 September 2023
JRC.F.5/WB/mt ARES(2023) 23-071

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

Reporting website	https://web.jrc.ec.europa.eu/ilcReportingWeb.
EU login	For help, see the Participant's guidelines
Password for reporting	«Part_key»
Questionnaire	https://ec.europa.eu/eusurvey/runner/GMFF-23_02_Questionnaire
Password	GMFF2302

Subject: **Instructions for GMFF-23/02, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* sheep feed and milled maize pasta**

Dear Dr «Surname»,

Thank you for participating to GMFF-23/02. In one of the following days you should receive two test materials, T1 and T2, containing 5 g (dry) of ground sample, sent at ambient temperature. **The vials should be stored in a fridge at approximately 4 ºC.**

- T1 is a ground feed test material declared as *containing GM material*.
- T2 is a ground food test material "*derived from a product that is not declared as containing GM material*".

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 – Sheep feed (feed) (5 g dry weight), declared as "*containing GM material"*:

- Identify all GM soybean events in this sample;
- Quantify the GM content in line with your routine procedures, for assessing the compliance of the sample with the applicable GMO legislation.

Note that the analysis results reported for T1 will be evaluated in terms of GM event identification, but no performance (*z*) scores will be determined for the quantitative results submitted. The latter will only be used for evaluating the correctness of the provided compliance statement. It is important to handle this sample as you would normally do for a similar test sample.

Test Item 2 – Milled maize pasta (food) (5 g dry weight), declared as "*derived from a product that is not declared as containing GM material*"

- Verify the presence of GM maize in this sample;
- Quantify the GM event identified (performance scores will be determined) and assess compliance of the sample 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**, as homogeneity of the test items has been demonstrated using this amount of sample.

When **reporting your results**:

- The default setting indicates "not tested" for all GM events; please change this into m/m % if reporting a quantitative (or a smaller/larger than) result, or to "present" or "absent" for reporting qualitative results; make sure you do this for all GM events indicated, as these results will be evaluated in the 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 (also for < LOD/LOQ);
- Report results with their expanded uncertainty (*U*) and coverage factor *k* (mandatory for the submission);
- Do not forget to select the technique used (default is "no technique").

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-23_02_Questionnaire, enter the password (see box below address line), and answer the questions of the survey. This survey includes questions on the analytical approaches used, and a statement on compliance to 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 10 November 2023. 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 4 °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 40-3-2 soybean in T1 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3
21	40.22	39.81	38.59
32	39.90	37.62	36.58
47	34.96	36.51	39.79
51	38.93	39.62	39.54
63	39.49	37.57	37.27
84	39.77	43.44	40.30
92	37.78	38.93	40.23
97	35.94	36.83	36.40
104	35.43	36.36	33.70
116	35.30	34.97	37.18
Mean	37.97		
s_x	1.87		
s_w	1.42		
S_s	1.68		
u^*	0.46		
σ_{pt}	10.45		
$0.3 * \sigma_{pt}$	3.14		
$S_s \leq 0.3^* \sigma_{pt}$	YES		
Assessment	Passed		

Homogeneity of 1507 maize in T2 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3
21	4.13	4.22	3.96
32	4.75	4.08	4.33
47	4.04	4.33	4.29
51	4.05	4.32	4.31
63	4.46	4.47	4.25
84	4.82	4.35	4.51
92	4.47	4.65	4.65
97	4.29	4.08	4.02
104	4.41	4.13	4.03
116	4.15	4.28	4.17
Mean	4.30		
s_x	0.17		
s_w	0.18		
S_s	0.14		
u^*	0.06		
σ_{pt}	1.16		
$0.3 * \sigma_{pt}$	0.35		
$S_s \leq 0.3^* \sigma_{pt}$	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 [14].

All values are in m/m %.

4.2 Stability

In the table below, the stability was assessed according to ISO 13528:2022 § B.5 [3].

Stability 1507 maize in T2 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	21	4.1	4.2	4.3	4.15
	116	4.2	4.3	4.2	
23	49	3.8	4.0	4.0	4.03
	95	4.4	3.9	4.1	

Slope ± 2 $SE_{(slope)}$ = -0.0052 ± 2 * 0.0042

Stability: passed

Annex 5. Evaluation of the reported screening results

Screening results reported for **T1** ('+': present, '–': absent; cells with results shown in green: correct, red: incorrect, white: not scored)

Type	Lab Code	p35S	tNOS	PAT	BAR	CP4-EPSPS	Ctp2-CP4-EPSPS	Cry1-Ab/Ac	pFMV	pNOS	t35S	nptII	p35S-pat	p35S-nptII	pCs VMV-pat	tE9	Agro	CMV	Other
NRL/625	L01	+	+	–	–		+												
NRL/625	L02	+	+	–	–		+												
NRL/625	L04	+	+	–				+	+										
OCL	L05	+	+	–	–		+	+	+		–								
OCL	L06	+	+						+										
NRL/625	L07	+	+	+	–		+												
NRL/625	L08	+	+	–			+	+								+			
NRL/625	L09	+	–	+	–	+			–	–		+							
OCL	L11	+	+	–	–				+										
NRL/120	L12																		
NRL/625	L13	+	+	–	–	+	+	+	+	–		–				+		–	yes
NRL/625	L14																		
NRL/625	L15	+	+	+	–		+										+		
NRL/625	L16	+	+	–												+			
NRL/625	L17	+	+	–	–		+	+											
NRL/625	L18	+	+		–		+	+					–						
OCL	L19	+	+																
NRL/625	L20	+	+		–		+	+	+				–						
OCL	L22																		
NRL/625	L23	+	+	–	–		+		+			–							
NRL/625	L24	+	+	–	–	+		+								+			
NRL/625	L25	+	+	–			+	+								+			–
NRL/625	L26	+	+	–	–		+		+							+			
NRL/625	L27	+	+		–		+		+				–					+	
NRL/625	L28	+	+	–															
OCL	L29	+	+	–			+	+								+			
NRL/625	L30	+	+	–	–											+			
NRL/625	L31	+	+	–			+										+		
NRL/625	L32	+	+	–	–	+		+	+										
NRL/625	L33	+	+	–	–		+	+	+				–						
NRL/625	L34	+		–															
NRL/625	L35	+	+	–	–		+	+	+							+			
NRL/120	L36	+	+	+	–		+		+				+		–		+		
OCL	L37	+	+	–	–											+			
NRL/120	L38	+	+	–	–		+	+	+							+			
NRL/625	L39	+	+	–				+	+							+			
NRL/120	L40	+	+																
NRL/120	L41			+	–		+								–		+		
NRL/120	L42	+	+	–			+												
NRL/120	L43	+	+	–	–		+		+	–									
NRL/120	L44			+			+												
OCL	L45	+	+	–	–	+	+	–	+							+			
NRL/625	L46	+	+	–	–		+	+	+			–					–	–	–
OCL	L47														–				
NRL/625	L48	+	+	–					+							+			
NRL/120	L49	+	+		–		+	+					+						
NRL/120	L50	+	+	–															
OCL	L51																		
NRL/120	L52	+	+	+	–		+					+	–						
NRL/625	L53	+	+	–	–		+		+	–	–	–				+	+		–
NRL/120	L54	+	+		–		+	+					–	–		+			–

Type	Lab Code	p35S	tNOS	PAT	BAR	CP4-EPSPS	Ctp2-CP4-EPSPS	Cry1-Ab/Ac	pFMV	pNOS	t35S	nptII	p35S-pat	p35S-nptII	pCs VMV-pat	tE9	Agro	CMV	Other
NRL/625	L56	+	+	-				+	+										
NRL/120	L57	+	+	+			+						+						
NRL/625	L58																		

Screening results reported for **T2** ('+': present, '–': absent; cells with results shown in green: correct, red: incorrect, white: not scored)

Type	Lab Code	p35S	tNOS	PAT	BAR	CP4-EPSPS	Ctp2-CP4-EPSPS	Cry1-Ab/Ac	pFMV	pNOS	t35S	nptII	p35S-pat	p35S-nptII	pCs VMV-pat	tE9	Agro	CMV	Other
NRL/625	L01	+	–	+	–		–												
NRL/625	L02	+	–	+	–		–												
NRL/625	L04	+	–	+				–	–										
OCL	L05	+	–	+	–		–	–	–		+								
OCL	L06	+	–						–										
NRL/625	L07	+	–	+	–		–		–										
NRL/625	L08	+		+			–	–								–			
NRL/625	L09	–	+	–	+	+			–	–		–							
OCL	L11	+	–	+	–														
NRL/120	L12	+	–	+	–		–												
NRL/625	L13	+	–	+	–	–	–	–	–	–		–				–		–	yes
NRL/625	L14																		
NRL/625	L15	+	–	+	–		–										–		
NRL/625	L16	+	–	+															
NRL/625	L17	+	–	+	–		–	–											
NRL/625	L18	+	–		–		–	–					+						
OCL	L19	+	–																
NRL/625	L20	+	–		–		–	–	–				+						
OCL	L21																		
OCL	L22																		
NRL/625	L23	+	–	+	–		–		+			–							
NRL/625	L24	+	–	+	–	–		–								–			
NRL/625	L25	+	–	+			–	–								–			
NRL/625	L26	+	–	+	–		–		–							–			
NRL/625	L27	+	–		–		–		–				+					+	
NRL/625	L28	+	–	+															
OCL	L29	+	–																
NRL/625	L30	+	–	+	–														
NRL/625	L31	+	–	+			–										–		
NRL/625	L32	+	–	+	–	–		+	–										
NRL/625	L33	+	–	+	–		–	–	–				+						
NRL/625	L34	+	–	+															
NRL/625	L35	+	–	+	–		–	–	–							–			
NRL/120	L36	+	–	+	–		–		–				+		–		–		
OCL	L37	+	–	+	–											–			
NRL/120	L38	+	–	+	–		–	–											
NRL/625	L39	+	–	+				–	–							–			
NRL/120	L40	+	–																
NRL/120	L41	+	–	+	–		–								–		–		
NRL/120	L42	+	–	+			–												
NRL/120	L43	+	–	+	–		–						+			–		–	
NRL/120	L44	+	–				–						+						
OCL	L45	+	–	+	–	–	–	+	–							–			

Type	Lab Code	p35S	tNOS	PAT	BAR	CP4-EPSPS	Ctp2-CP4-EPSPS	Cry1-Ab/Ac	pFMV	pNOS	t35S	nptII	p35S-pat	p35S-nptII	pCs VMV-pat	tE9	Agro	CMV	Other
NRL/625	L46	+	–	+	–		–	–	–			–					–	–	
OCL	L47	+	–	+													–	–	
NRL/625	L48	+	+	+			–		–							–			
NRL/120	L49	+	–		–		–						+						
NRL/120	L50	+	–	+															
OCL	L51																		
NRL/120	L52	+	–	+	–		–					+	+						
NRL/625	L53	+	–	+	–		–		–	–	+	–				–	–		
NRL/120	L54	+	–		–		–	–					+	–		–			
NRL/625	L56	+	–	+				–	–										
NRL/120	L57	+	–	+	–		–						+						
NRL/625	L58	+	–																

Annex 6. Results and laboratory performance

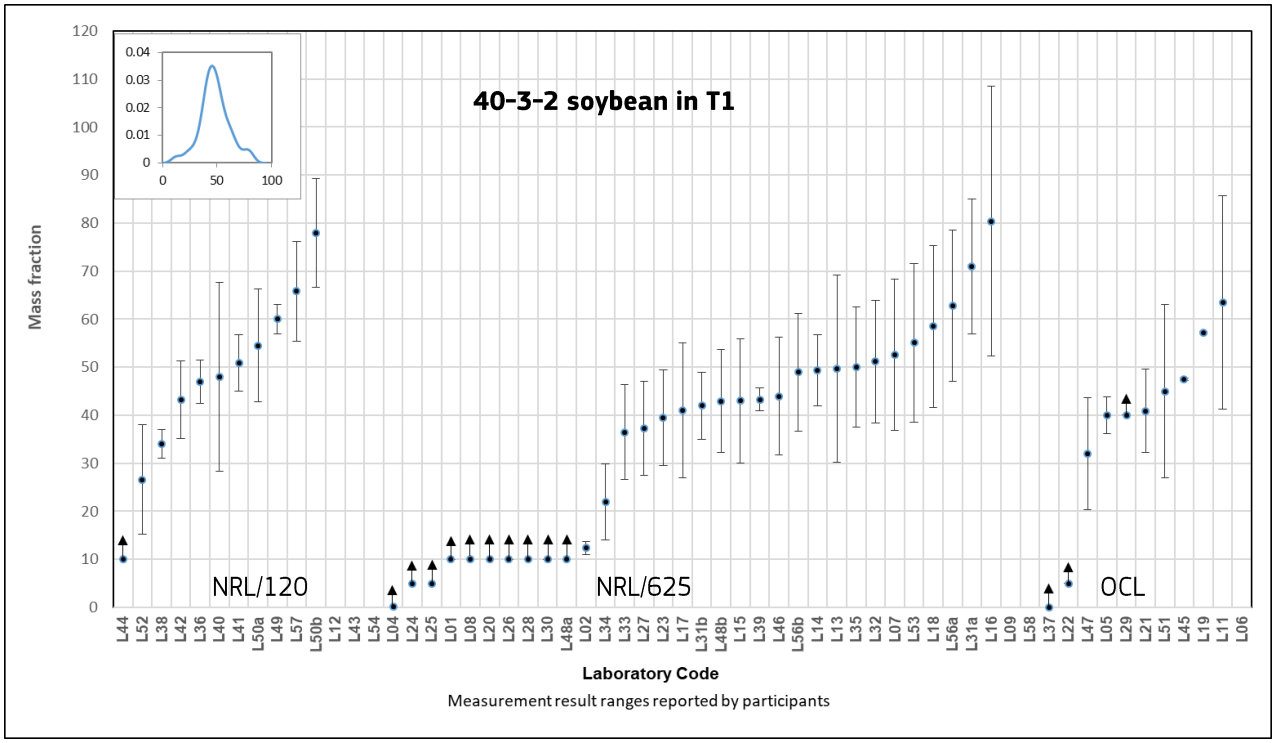
- ID = GM event identification (D = detected, ND = not detected, NT = not tested)
- For T1: values in columns 'xi-U' and '>0.9 %' were calculated by the PT coordinator to help compliance evaluation
- For T2: The PT coordinator set the measurement uncertainty $u(x_i)$ to zero when no expanded uncertainty was reported
- For T2: The PT coordinator set k = 1.73 when no coverage factor (k) was reported
- For T2: Performance scores (z and ζ): satisfactory, questionable, unsatisfactory
- For T2: 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: $u_{rel}(x_i) > \sigma_{pt,\%}$; NP = not provided

40-3-2 soybean in T1 (all values in m/m %)

Type	LabCode		x_i	U	k	Technique	ID	x_i-U > 0.9 %
NRL/625	L01	>	10			qPCR	D	Yes
NRL/625	L02	=	12.36	1.351	2	qPCR	D	Yes
NRL/625	L04	>	0.1				D	[a]
OCL	L05	=	40	3.78	2	dPCR	D	Yes
OCL	L06					qPCR	ND	
NRL/625	L07	=	52.6	15.78	2	qPCR	D	Yes
NRL/625	L08	>	10			qPCR	D	Yes
NRL/625	L09						NT	
OCL	L11	=	63.45	22.21	2	qPCR	D	Yes
NRL/120	L12						NT	
NRL/625	L13	=	49.68	19.47	2	qPCR	D	Yes
NRL/625	L14	=	49.33	7.44	2	qPCR	D	Yes
NRL/625	L15	=	43	12.9	2	qPCR	D	Yes
NRL/625	L16	=	80.4	28.14	2	qPCR	D	Yes
NRL/625	L17	=	41	14	2	qPCR	D	Yes
NRL/625	L18	=	58.5	16.9	2	qPCR	D	Yes
OCL	L19	=	57.2	0.27	2	qPCR	D	Yes
NRL/625	L20	>	10			qPCR	D	Yes
OCL	L21	=	40.92	8.69	2	qPCR	D	Yes
OCL	L22	>	5			qPCR	D	Yes
NRL/625	L23	=	39.44	9.92	2	qPCR	D	Yes
NRL/625	L24	>	5			qPCR	D	Yes
NRL/625	L25	>	5			qPCR	D	Yes
NRL/625	L26	>	10			qPCR	D	Yes
NRL/625	L27	=	37.22	9.81	2	qPCR	D	Yes
NRL/625	L28	>	10			qPCR	D	Yes
OCL	L29	>	40			qPCR	D	Yes
NRL/625	L30	>	10			qPCR	D	Yes
NRL/625	L31a	=	71	14	2	qPCR	D	Yes
NRL/625	L31b	=	42	7	2.23	dPCR	D	Yes
NRL/625	L32	=	51.18	12.8	2	qPCR	D	Yes
NRL/625	L33	=	36.46	9.86	2.78	qPCR	D	Yes
NRL/625	L34	=	22	7.9	1.73	qPCR	D	Yes
NRL/625	L35	=	50	12.5	2	qPCR	D	Yes
NRL/120	L36	=	47.02	4.55	3.18	dPCR	D	Yes

Type	LabCode		x_i	U	k	Technique	ID	x_i-U > 0.9 %
OCL	L37	>	0.05			qPCR	D	
NRL/120	L38	=	34	3	2	dPCR	D	Yes
NRL/625	L39	=	43.3	2.34	2	qPCR	D	Yes
NRL/120	L40	=	48.05	19.65	2	dPCR	D	Yes
NRL/120	L41	=	50.9	5.8	2	dPCR	D	Yes
NRL/120	L42	=	43.24	8.1	2	qPCR	D	Yes
NRL/120	L43					qPCR	D	
NRL/120	L44	>	10			qPCR	D	Yes
OCL	L45	=	47.52	0.3	2		D	Yes
NRL/625	L46	=	43.99	12.32	2	qPCR	D	Yes
OCL	L47	=	32	11.74	2	qPCR	D	Yes
NRL/625	L48a	>	10			qPCR	D	Yes
NRL/625	L48b	=	42.97	10.74	2	dPCR	D	Yes
NRL/120	L49	=	60	3.1	2	qPCR	D	Yes
NRL/120	L50a	=	54.54	11.74	2	dPCR	D	Yes
NRL/120	L50b	=	77.91	11.34	2	qPCR	D	Yes
OCL	L51	=	44.98	18	2	qPCR	D	Yes
NRL/120	L52	=	26.58	11.43	2	qPCR	D	Yes
NRL/625	L53	=	55.1	16.5	2	qPCR	D	Yes
NRL/120	L54					qPCR (QL)	D	
NRL/625	L56a	=	62.86	15.72	2	qPCR	D	Yes
NRL/625	L56b	=	48.98	12.24	2	dPCR	D	Yes
NRL/120	L57	=	65.8	10.4	2	qPCR	D	Yes
NRL/625	L58						NT	

[a] The lab commented: *When product is labelled as containing GMOs in routine analysis, detection of authorised GM events above the LOQ is considered sufficient to assess compliance and no quantification is performed*

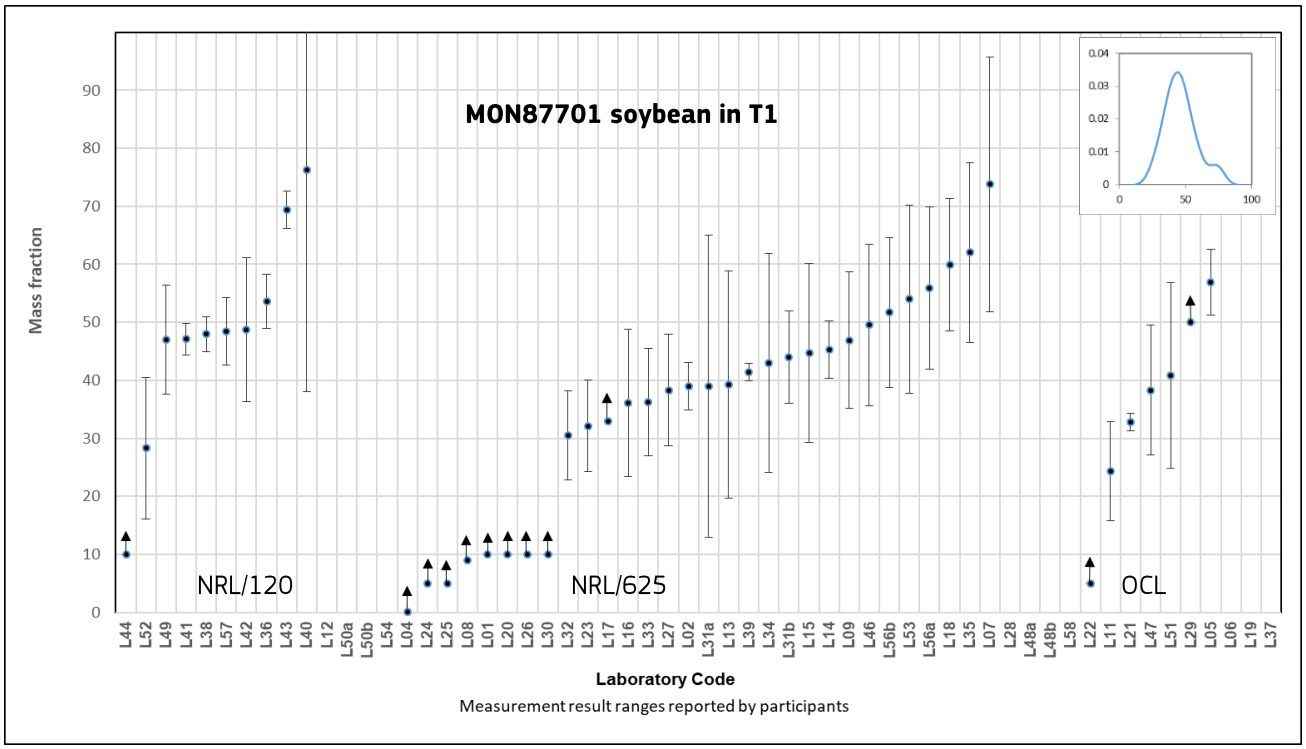


MON87701 soybean in T1 (all values in m/m %)

Type	LabCode	x_i	±	k	Technique	ID	x_i-U > 0.9 %
NRL/625	L01	> 10			qPCR	D	Yes
NRL/625	L02	= 38.98	4.112	2	qPCR	D	Yes
NRL/625	L04	> 0.1				D	[a]
OCL	L05	= 56.9	5.64	2	dPCR	D	Yes
OCL	L06				qPCR	D	
NRL/625	L07	= 73.82	21.98	2	qPCR	D	Yes
NRL/625	L08	> 9			qPCR	D	Yes
NRL/625	L09	= 46.92	11.73	2		D	Yes
OCL	L11	= 24.39	8.54	2	qPCR	D	Yes
NRL/120	L12					NT	
NRL/625	L13	= 39.3	19.54	1.65	qPCR	D	Yes
NRL/625	L14	= 45.34	4.95	2	qPCR	D	Yes
NRL/625	L15	= 44.7	15.4	2	qPCR	D	Yes
NRL/625	L16	= 36.13	12.65	2	qPCR	D	Yes
NRL/625	L17	> 33			qPCR	D	Yes
NRL/625	L18	= 60	11.4	2	qPCR	D	Yes
OCL	L19					NT	
NRL/625	L20	> 10			qPCR	D	Yes
OCL	L21	= 32.85	1.47	2	qPCR	D	Yes
OCL	L22	> 5			qPCR	D	Yes
NRL/625	L23	= 32.16	7.91	2	qPCR	D	Yes
NRL/625	L24	> 5			qPCR	D	Yes
NRL/625	L25	> 5			qPCR	D	Yes
NRL/625	L26	> 10			qPCR	D	Yes
NRL/625	L27	= 38.33	9.58	2	qPCR	D	Yes
NRL/625	L28					NT	
OCL	L29	> 50			qPCR	D	Yes
NRL/625	L30	> 10			qPCR	D	Yes
NRL/625	L31a	= 39	26	2	qPCR	D	Yes
NRL/625	L31b	= 44	8	2.45	dPCR	D	Yes
NRL/625	L32	= 30.51	7.63	2	qPCR	D	Yes
NRL/625	L33	= 36.21	9.24	2.78	qPCR	D	Yes
NRL/625	L34	= 43	18.9	1.73	qPCR	D	Yes
NRL/625	L35	= 62.05	15.51	2	qPCR	D	Yes
NRL/120	L36	= 53.65	4.65	3.18	dPCR	D	Yes
OCL	L37					D	
NRL/120	L38	= 48	3	2	dPCR	D	Yes
NRL/625	L39	= 41.44	1.52	2	qPCR	D	Yes
NRL/120	L40	= 76.3	38.27	2	dPCR	D	Yes
NRL/120	L41	= 47.1	2.7	2	dPCR	D	Yes
NRL/120	L42	= 48.8	12.4	2	qPCR	D	Yes
NRL/120	L43	= 69.42	3.2	2.57	qPCR	D	Yes
NRL/120	L44	> 10			qPCR	D	Yes
OCL	L45					ND	

Type	LabCode	x_i	±	k	Technique	ID	x_i-U > 0.9 %
NRL/625	L46	= 49.56	13.88	2	qPCR	D	Yes
OCL	L47	= 38.3	11.21	2	qPCR	D	Yes
NRL/625	L48a					D	
NRL/625	L48b					D	
NRL/120	L49	= 47	9.4	2	dPCR	D	Yes
NRL/120	L50a					ND	
NRL/120	L50b					ND	
OCL	L51	= 40.81	16	2	qPCR	D	Yes
NRL/120	L52	= 28.31	12.17	2	qPCR	D	Yes
NRL/625	L53	= 54	16.2	2	qPCR	D	Yes
NRL/120	L54				qPCR (QL)	D	
NRL/625	L56a	= 55.91	13.98	2	qPCR	D	Yes
NRL/625	L56b	= 51.69	12.92	2	dPCR	D	Yes
NRL/120	L57	= 48.4	5.8	2	qPCR	D	Yes
NRL/625	L58					NT	

[a] When product is labelled as containing GMOs in routine analysis, detection of authorised GM events above the LOQ is considered sufficient to assess compliance and no quantification is performed

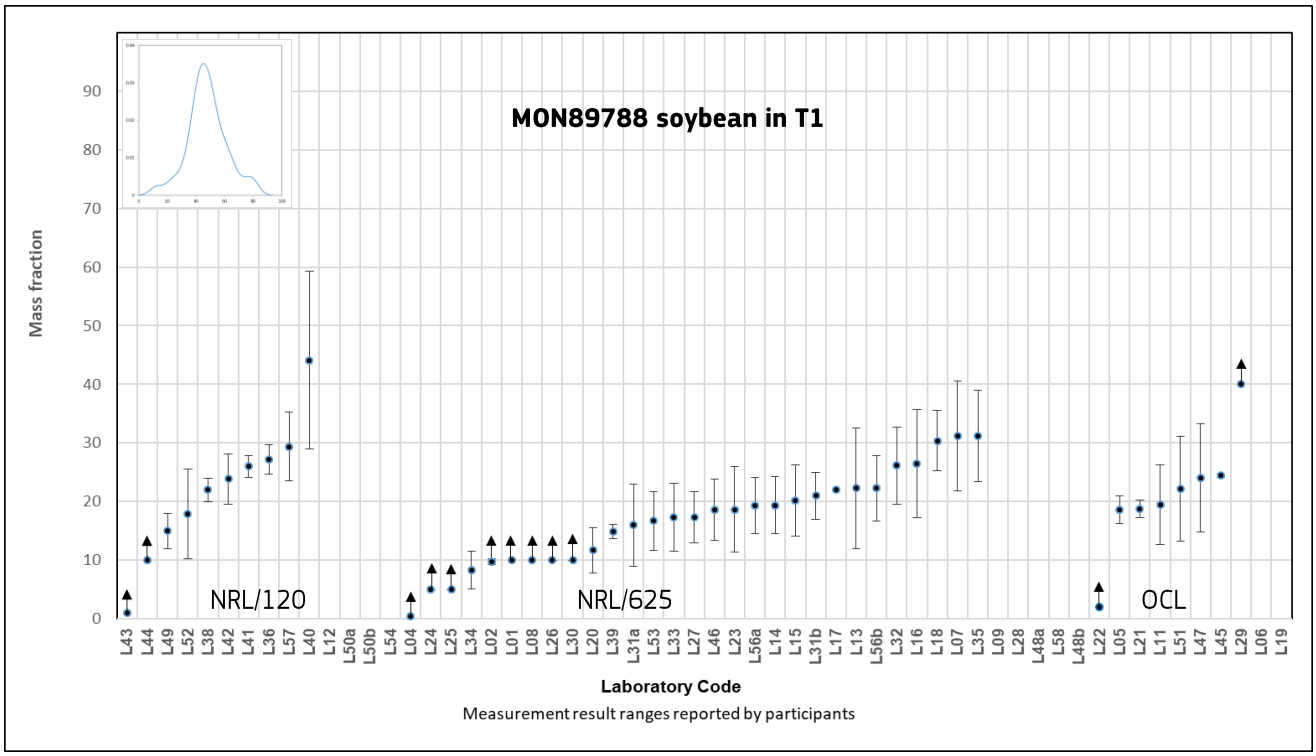


MON89788 soybean in T1 (all values in m/m %)

Type	LabCode		x_i	±	k	Technique	ID	x_i-U > 0.9 %
NRL/625	L01	>	10			qPCR	D	Yes
NRL/625	L02	=	9.76	0.529	2	qPCR	D	Yes
NRL/625	L04	>	0.4				D	[a]
OCL	L05	=	18.6	2.38	2	dPCR	D	Yes
OCL	L06					qPCR	D	
NRL/625	L07	=	31.23	9.36	2	qPCR	D	Yes
NRL/625	L08	>	10			qPCR	D	Yes
NRL/625	L09						NT	
OCL	L11	=	19.45	6.81	2	qPCR	D	Yes
NRL/120	L12						NT	
NRL/625	L13	=	22.28	10.28	1.65	qPCR	D	Yes
NRL/625	L14	=	19.37	4.87	2	qPCR	D	Yes
NRL/625	L15	=	20.2	6.1	2	qPCR	D	Yes
NRL/625	L16	=	26.41	9.24	2	qPCR	D	Yes
NRL/625	L17	>	22			qPCR	D	Yes
NRL/625	L18	=	30.4	5.1	2	qPCR	D	Yes
OCL	L19						NT	
NRL/625	L20	=	11.7	3.86	2	qPCR	D	Yes
OCL	L21	=	18.77	1.49	2	qPCR	D	Yes
OCL	L22	>	2			qPCR	D	Yes
NRL/625	L23	=	18.62	7.3	2	qPCR	D	Yes
NRL/625	L24	>	5			qPCR	D	Yes
NRL/625	L25	>	5			qPCR	D	Yes
NRL/625	L26	>	10			qPCR	D	Yes
NRL/625	L27	=	17.32	4.33	2	qPCR	D	Yes
NRL/625	L28						NT	
OCL	L29	>	40			qPCR	D	Yes
NRL/625	L30	>	10			qPCR	D	Yes
NRL/625	L31a	=	16	7	2.31	qPCR	D	Yes
NRL/625	L31b	=	21	4	3.18	dPCR	D	Yes
NRL/625	L32	=	26.12	6.53	2	qPCR	D	Yes
NRL/625	L33	=	17.3	5.79	2.78	qPCR	D	Yes
NRL/625	L34	=	8.3	3.2	1.76	qPCR	D	Yes
NRL/625	L35	=	31.25	7.81	2	qPCR	D	Yes
NRL/120	L36	=	27.21	2.53	3.18	dPCR	D	Yes
OCL	L37						NT	
NRL/120	L38	=	22	2	2	dPCR	D	Yes
NRL/625	L39	=	14.9	1.21	2	qPCR	D	Yes
NRL/120	L40	=	44.14	15.18	2	dPCR	D	Yes
NRL/120	L41	=	26	1.9	2	dPCR	D	Yes
NRL/120	L42	=	23.88	4.3	2	qPCR	D	Yes
NRL/120	L43	>	1			qPCR	D	Yes
NRL/120	L44	>	10			qPCR	D	Yes
OCL	L45	=	24.42	0.3	2	qPCR	D	Yes

Type	LabCode		x_i	±	k	Technique	ID	x_i-U > 0.9 %
NRL/625	L46	=	18.6	5.21	2	qPCR	D	Yes
OCL	L47	=	24	9.24	2	qPCR	D	Yes
NRL/625	L48a						D	
NRL/883	L48b						D	
NRL/120	L49	=	15	3	2	dPCR	D	Yes
NRL/120	L50a						ND	
NRL/120	L50b						ND	
OCL	L51	=	22.17	9	2	qPCR	D	Yes
NRL/120	L52	=	17.86	7.68	2	qPCR	D	Yes
NRL/625	L53	=	16.7	5.01	2	qPCR	D	Yes
NRL/120	L54					qPCR (QL)	D	
NRL/625	L56a	=	19.31	4.83	2	qPCR	D	Yes
NRL/625	L56b	=	22.31	5.58	2	dPCR	D	Yes
NRL/120	L57	=	29.4	5.8	2	qPCR	D	Yes
NRL/625	L58						NT	

[a] When product is labelled as containing GMOs in routine analysis, detection of authorised GM events above the LOQ is considered sufficient to assess compliance and no quantification is performed

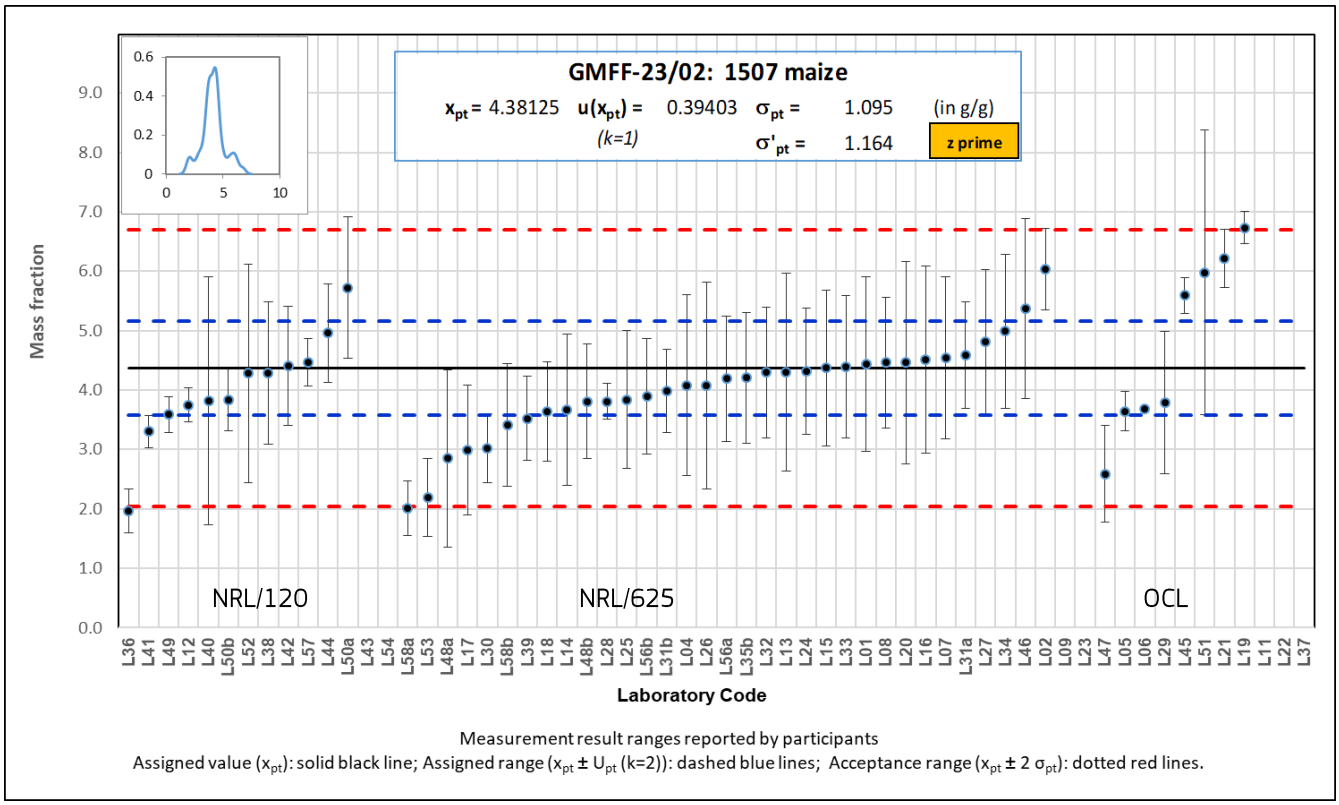


1507 maize in T2

Evaluation parameters: x_{pt} = 4.381 ; $u(x_{pt})$ = 0.394 ; σ_{pt} = 1.095 (all values in m/m %)

Type	LabCode	ID	= or </>	x_i	U	k	Technique	z prime	z score	unc.
NRL/625	L01	D	=	4.45	1.47	2	qPCR	0.06	0.08	a
NRL/625	L02	D	=	6.05	0.687	2	qPCR	1.43	3.19	b
NRL/625	L04	D	=	4.09	1.52	2		-0.25	-0.34	a
OCL	L05	D	=	3.65	0.33	2	dPCR	-0.63	-1.71	b
OCL	L06	D	=	3.7			qPCR	-0.59	-1.73	NP
NRL/625	L07	D	=	4.55	1.36	2	qPCR	0.14	0.21	a
NRL/625	L08	D	=	4.47	1.1	2	qPCR	0.08	0.13	a
NRL/625	L09	NT								
OCL	L11	NT								
NRL/120	L12	D	=	3.76	0.28	2.2	dPCR	-0.53	-1.50	b
NRL/625	L13	D	=	4.31	1.67	2	qPCR	-0.06	-0.08	a
NRL/625	L14	D	=	3.68	1.27	2	qPCR	-0.60	-0.94	a
NRL/625	L15	D	=	4.38	1.31	2	qPCR	0.00	0.00	a
NRL/625	L16	D	=	4.52	1.58	2	qPCR	0.12	0.16	a
NRL/625	L17	D	=	3	1.1	2	qPCR	-1.19	-2.04	a
NRL/625	L18	D	=	3.65	0.84	2	qPCR	-0.63	-1.27	a
OCL	L19	D	=	6.74	0.27	2	qPCR	2.03	5.66	b
NRL/625	L20	D	=	4.47	1.7	2	qPCR	0.08	0.09	a
OCL	L21	D	=	6.22	0.49	2	qPCR	1.58	3.96	b
OCL	L22	ND								
NRL/625	L23	NT								
NRL/625	L24	D	=	4.33	1.06	2	qPCR	-0.04	-0.08	a
NRL/625	L25	D	=	3.85	1.16	2	qPCR	-0.46	-0.76	a
NRL/625	L26	D	=	4.09	1.74	2	qPCR	-0.25	-0.30	a
NRL/625	L27	D	=	4.82	1.21	2	qPCR	0.38	0.61	a
NRL/625	L28	D	=	3.82	0.3	2	qPCR	-0.48	-1.33	b
OCL	L29	D	=	3.8	1.2	2	qPCR	-0.50	-0.81	a
NRL/625	L30	D	=	3.03	0.58	2	qPCR	-1.16	-2.76	a
NRL/625	L31a	D	=	4.6	0.9	2	qPCR	0.19	0.37	a
NRL/625	L31b	D	=	4	0.7	2.26	dPCR	-0.33	-0.76	b
NRL/625	L32	D	=	4.31	1.1	2	qPCR	-0.06	-0.11	a
NRL/625	L33	D	=	4.4	1.2	2	qPCR	0.02	0.03	a
NRL/625	L34	D	=	5	1.3	2	qPCR	0.53	0.81	a
NRL/625	L35	D	=	4.22	1.1	2	qPCR	-0.14	-0.24	a
NRL/120	L36	D	=	1.98	0.37	2.57	dPCR	-2.06	-5.72	b
OCL	L37	NT								
NRL/120	L38	D	=	4.3	1.2	2	dPCR	-0.07	-0.11	a
NRL/625	L39	D	=	3.53	0.71	2	qPCR	-0.73	-1.61	a
NRL/120	L40	D	=	3.83	2.09	2	dPCR	-0.47	-0.49	c
NRL/120	L41	D	=	3.31	0.27	2	dPCR	-0.92	-2.57	b
NRL/120	L42	D	=	4.42	1	2	qPCR	0.03	0.06	a
NRL/120	L43	D					qPCR			

Type	LabCode	ID	= or </>	x_i	U	k	Technique	z prime	z score	unc.
NRL/120	L44	D	=	4.97	0.83	3.18	qPCR	0.51	1.25	b
OCL	L45	D	=	5.6	0.3	2	qPCR	1.05	2.89	b
NRL/625	L46	D	=	5.38	1.51	2	qPCR	0.86	1.17	a
OCL	L47	D	=	2.6	0.82	2	qPCR	-1.53	-3.13	a
NRL/625	L48a	D	=	2.86	1.49	2	qPCR	-1.31	-1.81	c
NRL/625	L48b	D	=	3.82	0.96	2	dPCR	-0.48	-0.90	a
NRL/120	L49	D	=	3.6	0.3	2	qPCR	-0.67	-1.85	b
NRL/120	L50a	D	=	5.73	1.19	2	qPCR	1.16	1.89	a
NRL/120	L50b	D	=	3.85	0.53	2	dPCR	-0.46	-1.12	b
OCL	L51	D	=	5.99	2.4	2	qPCR	1.38	1.27	a
NRL/120	L52	D	=	4.29	1.84	2	qPCR	-0.08	-0.09	a
NRL/625	L53	D	=	2.2	0.66	2	qPCR	-1.87	-4.24	a
NRL/120	L54	D					qPCR (QL)			
NRL/625	L56a	D	=	4.2	1.05	2	qPCR	-0.16	-0.28	a
NRL/625	L56b	D	=	3.9	0.97	2	dPCR	-0.41	-0.77	a
NRL/120	L57	D	=	4.47	0.4	2	qPCR	0.08	0.20	b
NRL/625	L58a	D	=	2.02	0.46	2	qPCR	-2.03	-5.18	a
NRL/625	L58b	D	=	3.42	1.03	2	dPCR	-0.83	-1.48	a



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	53	55
No	2*	0
No Answer	0	0

*L12 and L58 did not analyse T1 because the sample matrix was out of the scope of the laboratories' accreditation

Are the methods used within the scope of accreditation of your laboratory under ISO/IEC 17025?

Method	Test Item	Flexible	Fixed	Some	No	Not applicable
DNA extraction method	T1	33	14	0	5	1
	T2	33	14	0	7	1
Qualitative GMO screening method(s)	T1&T2	34	12	5	3	1
Qualitative event-specific identification method(s)	T1&T2	29	7	16	3	0
Quantitative event-specific GMO method(s): qPCR	T1	29	7	9	3	9
	T2	26	6	4	10	10
Quantitative event-specific GMO method(s): dPCR	T1	6	4	1	6	38
	T2	6	4	1	6	39

Further explanation on the work not done under accreditation

Some recently approved GM events not yet in scope of accreditation
GMO screening methods and quantitative event specific methods are our flexible accredited methods.
The events A2704-12 and A5547-127 are not yet in the scope of our accreditation.
The accreditation of the laboratory refers exclusively to the examination of foodstuffs.
Not yet flexible scope
Sample T2 is not under our accreditation scope (food, but we have accreditation for feed) (2x)
Previous methods are in validation/verification for accreditation. Recent methods in verification for implementation in the laboratory.
dPCR will be in the scope of accreditation from 2024; it is in the auditing process
Normally we are accredited for feed and seed, not food. But the flexibility should be enough for including.
T1: Successfully inspected by the German Accreditation Body (DAkkS) but accreditation not yet completed
The laboratory is accredited to ISTA Accreditation Standard (2x)
We are currently verifying an additional number of GM events (e.g. LOD/LOQ determination), in function of addition to our flexible scope for QN GMO analysis based on qPCR. For dPCR we will start with preparing extension of accredited scope (QN GMO analysis based on ddPCR) in 2024.

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	3	4
CTAB method with 2% CTAB in lysis buffer	18	19
Maxwell RSC PureFood GMO and authentication kit	6	6
Maxwell RSC/LEV Plant DNA kit	1	1
NucleoSpin Food	16	14
NucleoSpin Plant	3	3
NucleoMag DNA Food	2	2
NucleoMag DNA Plant	0	0
GeneSpin	5	4
Wizard genomic DNA purification kit	0	0
Qiagen DNeasy Plant	1	1
Qiagen DNeasy Mericon Food	4	2
Qiagen Blood and Tissue kit	1	1
Biotecon Foodproof	2	2
SDS	1	2
Speedtools Food DNA extraction kit (Biotools)	1	1
Generon Ion Force	0	0
Eurofins DNAExtractor cleaning column	2	2
Promega Wizard DNA clean-up resin	0	1
Qiagen QIAQuick	1	3
Qiagen Genomic-Tip 20/G	0	0

NucleoSpin gDNA clean-up	0	0
Zymo OneStep PCR inhibitor Removal kit	2	2
Qiaex II purification kit	1	1

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

Increased amount of sample (0.5 g) and volume of lysis buffer (NSF)
Low concentration of DNA in both samples so we had to perform isolation twice. (GeneSpin)
For T2 the lysate is applied twice to the spin column (NSF)
T2 - longer incubation time, T1 - NucleoSpin filters cat. no 740 606, using the washing CQW buffer twice
We used SureFood PREP Advanced DNA extraction kit.
Pre-step with Lysing Matrix A. Qiagen Blood and Tissue kit: The solutions were added 4 times the volume indicated in the kit.
RNase treatment
The CTAB method was combined with Maxwell 16 FFS Nucleic Acid Extraction System, custom (Promega)
T1: A CTAB lysis was performed prior to the DNA extraction with MN Food Kit T2: SureFood Prep Advanced Kit
CTAB lysis with magnetic bead clean up (NucleoMag Plant)
Lysis with CTAB buffer (NSF)

Select the quality control tests performed on the extracted DNA

Answer	**T1**	**T2**
No quality control tests done	1	3
We confirm that the OD ratio @ 260/280 nm is between 1.8 and 2.0	27	30
We confirm that the OD ratio @ 260/230 nm is > 2.0	8	9
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)	6	6
We analysed two or more dilutions of the DNA and compared the qPCR results	17	17
An external positive control was added to the unknown samples	7	7
Other	1	1

Provide further clarification on the approach used for DNA quality analysis and the outcome

Ratio 260/230 on T1 is <2 when using the selected kits. (2x)
260/230 and 260/280 ratio's are based on undiluted DNA. Inhibition tests were performed during quantification.
QC 260/280 and 260/230 checked but not always in the range of specifications above (mean values : T1 : 260/280 = 1.7 ; 260/230 = 1.8 ; T2 : 260/280 = 1.7 ; 260/230 = 2.4)
T1: OD ratio @ 260/280 nm is 2,06/2,08
We inspect the curves for samples after qPCR.
T1: Absence of inhibition was tested by two dilutions of the DNA on three reference gene targets
External positive control, analyzed in parallel

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	49	0	54	1	2
tNOS	47	1	2	52	2
PAT	8	35	42	1	12
BAR	1	30	1	31	24
CP4-EPSPS	5	0	1	4	52
Ctp-CP4-EPSPS	0	0	0	0	57
Ctp2-CP4-EPSPS	32	0	0	33	23
Cry1Ab/Ac	19	1	2	16	37
Cry1Ab	0	0	0	0	57
pFMV	21	1	1	19	34
pNOS	0	4	0	3	53
t35S	0	2	2	0	55
nptII	2	4	1	5	51
p35S-pat	3	6	11	0	46
p35S-nptII	0	1	0	1	56
pCsVMV-pat	0	3	0	2	54
tE9	17	0	0	13	39
Agrobacterium border seq.	6	1	0	8	49
CaMV	1	2	1	4	52
Other	0	4	0	4	53

Further details on other screening targets

barstar not detected, cotton not detected, rice actin 1 not detected, canola T1 detected, canola T2 not detected, P-rice actin not detected, Vip3A not detected, maize detected, pea not detected, PSsuAra T1 detected, PSsuAra T2 not detected, rice not detected, gat/T-pinII not detected, soy T1 detected, soy T2 not detected, Cry1F T1 not detected, Cry1F T2 detected, T-g7 not detected, CV127 not detected, alfalfa T1 detected, alfalfa T2 not detected, DAS40278 not detected, DP305423 not detected, VCO1981 not detected.
Agroborder II used
SAMS, AINT
T1: AgroBorder II positive
P-nos-nptII
T-ORF23

GMO identification (qualitative): for each GM event, its presence/absence/not tested has to be indicated in the online reporting form (MILC). If applicable, indicate here any other GM event-specific tests done for T1 or T2 (and the result).

T1: 356043 absent; T2: 98140, VCO01981-5, Bt176 absent
For T2 identification of MON863 - result absent
T1: soybean 356043 (NEG), T2: maize 98140 (NEG), maize VCO1981 (NEG).
T1 events tested (result neg.): 305423; CV127; Mon87708 (traces); MON87769; A2704-12; A5547 (traces); MON87705; FG72; SYHT0H2;GMB151; Mon87751. T2 events tested (result): DAS40278 (neg.); 59122 (neg.); MON810 (traces, neg.); DP4114 (neg.); T25 (neg.); MON95379 (neg.); MON87419 (neg.).
T1: 356043 - absent; T2: 98140 and VCO-01981-5 - absent
T1 - DP356043; T2 - 98140, BT176, LY038, MON863.
T1: tested and not detected: DP 356043 T2: tested and not detected: MON 863; CBH-351-2; LY038; MON 98140; VCO 1981-5
T1: DP356043: Absent (qPCR) T2: LY038 (absent, qPCR); 98140 (absent, qPCR)
T1 356043 absent, T2: 356043 absent, VCO -01981-5 absent, T14 absent
T1: 356043 Absent; T2: VCO1981 Absent, MON863 Absent, Bt176 Absent, 98140 Absent
for T2 we also tested sample for VCO-1981, result negative, and 98140, result negative.
T1: DP356043, IND410, MON87712, DBN9004, MON87754 T2: DP98140, HCEM485, LY038, TC6275 All results - absent
T1 all absence: FG72, MON87769, GMB151, 305423, 356043, CV127, MON87705, MON87708, MON87751; T2 all absence: T25, DP-004114, MON87403, MON87429, DO98140, MON810, DAS-59122, MZIR098, MZHG0JG, MON87419, LY038, DAS-40278, VCO-01981; T2 MON95379 not tested because reason of time
Remarks on qualitative MON810 detection for T2: We reported as "absent" in the reporting website. However we observed a plot (<LOD) in each extract (diluted as well as undiluted sample). Possibly, due to a combination of inhibition in the undiluted sample and necessary dilution of the sample, we could not confirm the suspectable low presence of MON810 in the sample.
E32 - LY038 - VCO1981-5 - Bt176 - 98140
LY038 - VCO1981-5

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

Test item	Reference target	Answers
T1 (sheep feed)	Soybean *Le1* (70 bp) - QT-TAX-GM-004	1
	Soybean *Le1* (74 bp) - QT-TAX-GM-002	41
	Soybean *Le1* (81 bp) - QT-TAX-GM-001	4
	Soybean *Le1* (102 bp) - QT-TAX-GM-003	0
	Soybean *Le1* (102 bp) - QT-TAX-GM-020	1
	Soybean *Le1* (105 bp) - QT-TAX-GM-009	1
	Soybean *Le1* (118 bp) - QT-TAX-GM-007	0
	Other	3
T2 (maize pasta)	Maize *hmg*	48
	Maize *Adh1* – 134/136 bp	7
	Maize *Adh1* – 70 bp	1
	Maize Invertase	0
	Other	1

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	40-3-2	JRC	ERM-BF410bp (100 %)	10
			ERM-BF410ep (10 %)	11
			ERM-BF410gk (10 %)	3
			ERM-BF410dk (1 %)	1
			ERM-BF410gn (1 %)	2
			ERM-BF410dn (1 %)	1
	MON87701	AOCS	0809-A2	13
			0809-A	18

	MON89788	AOCS	0906-B	16
			0906-B2	14
T2	1507	JRC	ERM-BF418d	34
			ERM-BF418 (series)	6

Note: 11 laboratories also indicated the use of ERM-BF413(gk) for quantification of MON810 in T2, and some of them reported results between 0.01 and 0.1 m/m %

When using digital PCR, which general system did you use?

Digital PCR system	Answers
Droplet dPCR, total	10
BioRad QX100	1
BioRad QX200	9
Chamber dPCR, total	2
Qiagen QIAcuity	2

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

No (3x)
For all dPCR-assays: Primer conc. 0.4 uM, probe conc. 0.2 uM. This concentrations are also applied for qPCR-assays (divergent from the EURL-GMFF reference methods). Reason: standardisation of dPCR assays
For improved discrimination of the droplet populations, the primers and probes were used at a final concentration of 630 nM (for primers) and 175 nM (for probes), respectively.
We use 500 nM of each primer and 100 nM of each probe
For better resolution the primer/probe concentrations were adjusted to 600/600 nmol and 200 nmol
yes: primers and probes according to the manufacturers recommendations: primers 900 µM; probes 250µM
yes, MON89788 (primer 900nM, probe 250nM); MON87701 (primer 500nM, probe 250nM)
Yes, we increased the final concentration in digital PCR method, following the supplier suggestion
For LEC and RRS (GTS40-3-2) we changed the primer and probe concentrations according a Reference Article: Bogozalec et al., 2019 (better separation of cluster bands). For the other systems we used the same concentration as in qPCR.
0.9 µM primers and 0.25 µM probes recommended by manufacturer

Indicate the conversion factor(s) used to convert your dPCR results from a GM copy number ratio to a GM mass fraction. The conversion factor recommended by the EURL GMFF is shown in bold.

Test item	GM event	Conversion factor	Answers
T1	40-3-2	**1.03**	6
		0.817	1
		0.973	1
		1	1
		1.06	1
	MON87701	**0.97**	3
		0.739	1
		0.95	1
		0.956	1
		1	1
		1.2	1
	MON89788	**0.98**	4
		0.931	1
		0.962	1
		1	1
T2	1507	**0.61**	9
		0.637	1
		0.659	1

What is the dynamic range of the GM event-specific methods used for this PT, determined during method validation/verification in your laboratory (expressed in m/m %)?

Test item	GM event	Lowest to highest point	Answers	Comments
T1	40-3-2	0.1 to 1	1	
		≤0.1 to 5-9	5	
		≤0.1 to ±10	15	
		≤0.1 to ±100	14	
		≥10 to <<100	2	10 to 100; 15.2 to 38.0
	MON87701	0.1 to 1	2	
		≤0.1 to 5-9	7	
		≤0.1 to ±10	7	
		≤0.1 to ±100	13	
		>>10 to <<100	1	16.1 to 40.1
	MON89788	0.1 to 1	3	
		≤0.1 to 5-9	3	
		≤0.1 to ±10	9	

		≤0.1 to ±100	13	
		>>10 to <<100	2	10.2 to 25.5
T2	1507	0.1 to 1	2	
		≤0.1 to 5-9	5	
		≤0.1 to ±10	15	
		≤0.1 to ±100	9	
		>>10 to <<100	0	
		other	2	2.4 to 6.1; 0.2 to 16.7

Were the GM % measured in the test items falling within the validated dynamic range of the methods (indicated in Q8)? Did you require to extend the dynamic range of some methods for these measurements? Why/why not?

Test result was over dynamic range in methods MON89788, MON87701 and 40-3-2. We did not extend the dynamic range and the result was given >"highest point of dynamic range". Our quality management instructions guide to express result that way.
The dynamic range of the method is determined in cp numbers for the event-specific and reference methods
Yes, the GM% measured in the samples fell within the validated dynamic range (of dPCR quantification)
EU reference methods are usually validated for the range of 0.1% to 5% GM content. In our laboratory, the acceptance criteria for the relevant thresholds of 0.9% and 0.1% are verified. It can be assumed that the methods in our laboratory are also valid up to the upper limit of the EU validation.
We did not change anything in the T2 sample because it is in the range. As for the t1 sample, with regard to routine work, no quantification was done because we have prescribed: "In the case when, during the qualitative determination, the difference between the Ct value of highly specific and transgenic DNA is less than 4.0, without further analysis we can conclude that the sample contains less of more than 10% transgenic DNA." which was shown in this case where the difference between Ct was 2- 3.
The % measures does not fall within the dynamic range. No extension of the dynamic range is needed because as it pertains to a relative quantification using a 100% CRM. The DNA amplification of both the transgene and the endogenous genes DNA occurred within the respective calibration curves.
Yes, the measured GM fraction in T2 was within the dynamic range of the method. No extension was necessary.
Q9a. No. Q9.b. No. the dynamic range (determined during in-house verifications methods) is based on a copy number approach and inspired by the recommendations mentioned in paragraph 2.3.2 of the document JRC95544-2015. We consider (at the moment) that the extension of the dynamic range is not a relevant option (especially regarding real samples tested in official controls).
The measured values were clearly above the verified dynamic range. From our point of view, a re-verification is not necessary, because - in-house verification data show a satisfactory performance of the method in the calibration range of 0.9 -10 %, - an interlaboratory validation study with satisfactory performance data is available, - it is not relevant (in this case) for checking results around thresholds of 0.1/0.9%, - for stacked-events the summation of the single events is in place and therefore exact values are not needed.
Values were out of the dynamic range of the validated methods
T1 : absolute values were within the dynamic range
In general we do not extend the dynamic range if the obtained result is sufficient to decide on compliance with the EU legislation. We would extend the dynamic range if the customer asks for precise results.
Yes, for all three soybean events; For MON810 GM target was outside dynamic range (lower than LOQ).
No. We did not consider it necessary regarding legal requirements.
No. The compliance of the sample with the GMO legislation can be assessed.
In T1 were not falling in the validated dynamic range. We report the GM content in this sample is larger than the upper limit of our validated dynamic range.
Yes, we needed to extend the dynamic range
The GM % measured in the test items were falling within the validated dynamic range. We did not need to extend the dynamic range.
T 1: For GTS-40-3-2, MON 89788 and MON 87701 the known dynamic range was exceeded as the measured percentage values of each soybean line was above the individual max. values (table 8). We did not extend the dynamic range by systematic experimental assessment as the measured values were way above the legal threshold of 0.9 %. Furthermore quantification with ddPCR inherently requires dilution of the measured DNA to a concentration that lies within the dynamic range of the ddPCR. This in turn leads to an accurate measurement of DNA copy numbers. T 2: For TC 1507 the measured value was within the dynamic range (table 8).
1-3: no; 4: yes We did not prepare new materials with more than 10% m/m to be fit for this PT. In practice, it will not be necessary to accurately determine the GM percentage above 10% m/m. Additionally, the applicability the method at least for a semi-quantitative estimation of the percentage above the dynamic range can be shown by the participation in this or similar PTs
No, the GM% measured in the test items are not in the validated dynamic ranges. Because of the lack of suitable CRMs in the most cases the correct determination of a dynamic range is not possible. The results are sure above the in house validated dynamic range and sure >0.9%. This is the needed information respectively to VO(EG) 1829/2003. A detailed declaration of the measured value is not demanded by VO(EG) 1829/2003.
The percentage of GM measured in the test items was not within the approved dynamic range of MON87701. This method required an extension of the dynamic range.
Only for the event TC1507. For events 40.3.2 and MON810, GM% were not within the validated dynamic range, but we inform that the value is up or below the range.
GTS 40-3-2 measurement is not falling in the dynamic range. We did not extend the dynamic range, because of labelling of the sample as "containing GMO". Therefore evaluation of the declaration did not need exact quantified values.

Our validations are normally done with 0,1 % , 1 % and 5 % materials - thus all soy events quantified were clearly above our dynamic range. We did not extend the dynamic range as the events detected were allowed on EU market and the sample was declared as contains GM. Our monitoring officials are only really interested in samples that would be labelled as non-GM and the m/m% is around labelling limit (0,9 %)
The GM %'s measured were falling within the validated dynamic range of the used methods (Absolute quantification with two calibration curves), so no extension of the dynamic range was required.

If the validated dynamic range of a method is having an upper limit of e.g. 10 m/m % GMO and the GM content in a sample is say 20 m/m %, which option best describes your approach for official control.

Options	Answers
We report that the GM content is larger than the upper limit of our validated dynamic range (e.g. > 10 m/m %)	24
We measure a dilution of the sample and make sure the Cq values fall within the calibration curves, then report the result for the undiluted sample (regardless of the validated range), e.g. 20 m/m %	13
We re-validate the method for an extended dynamic range in order to cover the GM content in the sample	4

Further comments

Response to previous question [reporting >range] is valid for delta Ct methods where the validated dynamic range is determined as % m/m
If a result is above the validated dynamic range, a comment is added to the result if appropriate.
We should have reported > 10 m/m % respectively > 5 m/m % but we reported the exact value instead
We do not use a dynamic range based on GM %. We use it with Cq values of the standard curve.
We report that the GM content is larger than the upper limit of our validated dynamic range (> 10 m/m % together with U from the method)
We used the official conversion factor for GTS 40-3-2 for our reported result. Analytically, we feel that this is not justified as the cf changes for this material as the concentration (in m/m%) changes. We also used the "old" cf from the CF-Guide Ver. 5 for 10% material (0,79 +- 0,14 m/m%), that we find more fitting. Doing so, we determine a value of 55 +-13 m/m%; k=2,2. We strongly support the idea of discussing this further.
We only analyze food samples in which GMOs are usually present in small quantities. Therefore, we have verified and accredited a method for the quantification of soybean 40-3-2 using 10% CRM (ERM-BF410ep, according to EURL validated protocol). For the purpose of the analysis of sample T1 (feed) in this PT I prepared a calibration curve from ERM-BF410bp (98.5%), as I saw that the contamination was higher than 10%. However, I also tested the T1 sample with a 10% calibrant but got a higher result (47.12%) compared to the reported result (36.46%). I also noticed that when I used 100% CRM for calibration, I got lower values for 1% CRM (0.78%).
Due to lack of CRMs covering the full range of 0.1 - 100 % m/m GMO, a validation of the dynamic range covering low (0.1 / 1 %) and high (> 50%) GMOs is difficult. We think the validation of the dynamic range higher than 5 % GMO is not essential when testing for compliance with the EU GMO legislation.
We make a mixture between the two noted answers: We measure a dilution but report the GM content e.g. >0.9%.
In routine analysis in our lab, none of the given possibilities are relevant so far, because such samples did not exist.
MON 87708 was present in only 1/4 technical replicates (we redid the quantification assay 3 times)
We don't have any dynamic range validated in dPCR method for the moment

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

Options	Answers
Using data obtained on routine samples (control charts)	5
Using data from in-house method validation or method verification	25
Following the bottom-up approach described in the GUM (assessing the uncertainties at every step)	3
Using the measurement uncertainty determined by the EURL GMFF from the interlaboratory trial used for method validation	8
Estimated as a fixed percentage of the result	12
Other	5

If selected other, please clarify

According to Application Note: "Use of the harmonised conversion factors to transform PCR results from the DNA copy number ratio domain into the mass fraction domain"
Derived from the standard deviation of the sample measurements/extracts (4x)
MU = 2 SD for all measurements + estimation of the bias on control sample (if available)
According Guidance document on Measurement Uncertainty for GMO Testing Laboratories 3rd Edition, chapt..2
Estimation of matrix-related uncertainties
We used MU determined by the sample measurement in some cases.
using data from in-house method validation by kit manufacturer
In not validated methods (used only on this PT rounds, not on routine samples) we estimate the uncertainty as a fixed percentage of the result.

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.

Additional comments and suggestions

We have followed our routine workflow. In case sample is labelled as containing GMO (usually it is specified GM soy, GM maize etc) all events that are authorised and found to be >LOQ of the method are not further quantified.
We would normally not find it relevant to test T1 for authorized GMO. Only for unauthorized GMOs. And that would normally be on feed raw material.
As regards question from point 5, sample is in line with the labelling ruler because it was labelled as containing GMOs.
Thanks for this interesting PT!
Thanks again!
Only our ministry can conclude about compliance

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