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EURL European Union Reference Laboratory for GM Food & Feed

Determination of GM Maize Event 59122 in Multigrain Bread Mix and GM Soybean Event CV127 in Soybean Powder

Report of the EURL GMFF Proficiency Test GMFF-22/02

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Contents

Abstract

European legislation on genetically modified organisms (GMOs) requests the monitoring of the presence of GMOs in food or feed by analytical tests. Such monitoring ensures that food and feed products on the market contain only authorised GMOs and that their presence is mentioned on the product label if the content is above a legally defined threshold. The analytical tests are carried out by laboratories designated for official controls by the EU Member States. To assess the uniform and reliable performance of these control laboratories proficiency tests (PTs) are organised by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) in line with Regulation (EU) 2017/625 on official controls. This report summarises the results of the PT "GMFF-22/02" for the determination of GMOs in multigrain bread and soybean flour. The evaluation of the results submitted by 63 laboratories confirms that most analytical laboratories are able to identify and quantify GMOs in food and feed samples.

Acknowledgements

BASF is acknowledged for kindly providing the ground CV127 seeds. The laboratories listed hereafter are acknowledged for their participation to the PT round.

Organisation	Country
AGES - Institute for Food Safety Vienna	AUSTRIA
Umweltbundesamt GmbH	AUSTRIA
CRA-W	BELGIUM
ILVO	BELGIUM
Sciensano	BELGIUM
Laboratory of SGS Bulgaria	BULGARIA
National Center of Public Health and Analysis	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
BioGEVES	FRANCE
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe	GERMANY
Thueringer Landesamt fuer Verbraucherschutz	GERMANY
LAVES-LVI BS/H	GERMANY
Landesamt für Verbraucherschutz Sachsen-Anhalt	GERMANY
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V	GERMANY
Institute for Hygiene and environment	GERMANY
LUFA Speyer	GERMANY
CVUA Freiburg	GERMANY
LTZ Augustenberg	GERMANY
Landeslabor Schleswig-Holstein	GERMANY
Federal Office of Consumer Protection and Food Safety (BVL)	GERMANY
Landeslabor Berlin Brandenburg	GERMANY
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GERMANY
AGROLAB LUFA GmbH	GERMANY
General Chemical State Laboratory (GCSL)	GREECE
Biomi Kft	HUNGARY
National Food Chain Safety Institute	HUNGARY
CREA Centro di Ricerca Difesa e Certificazione	ITALY
Istituto Zooprofilattico Sperimentale Lazio e Toscana	ITALY
Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna	ITALY
ATS Milano Citta' Metropolitana	ITALY
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta	ITALY
Institute of Food Safety, Animal Health and Environment „BIOR"	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
Laboratoire National de Santé	LUXEMBOURG
Wageningen Food Safety Research (WFSR)	NETHERLANDS
Wojewódzki Inspektorat Weterynarii w Opolu	POLAND
National Veterinary Research institute	POLAND
Plant Breeding and Acclimatization Institute NRI	POLAND
INIAV	PORTUGAL
Institute of Diagnosis and Animal Health	ROMANIA
LCCSMS	ROMANIA
SP Laboratorija a.d.	SERBIA
Central Control and Testing Institute of Agriculture, Bratislava	SLOVAKIA
State Veterinary and Food Institute, VFI in Dolny Kubin	SLOVAKIA
National Institute of Biology	SLOVENIA
Centro Nacional de Alimentación	SPAIN
Laboratorio de Control Oficial Agrolimentario y Agroganadero de Sevilla	SPAIN
Laboratorio Central de Veterinaria	SPAIN
SeLyC	SPAIN
Laboratorio Agroalimentario de Navarra	SPAIN
Laboratorio Arbitral Agroalimentario - MAPA	SPAIN
Swedish Food Agency - Livsmedelsverket	SWEDEN
Federal Food Safety and Veterinary Office FSVO	SWITZERLAND
National Food Reference Laboratory	TURKEY
Ankara Food Control Laboratory (ANKARA GIDA KONTROL LABORATUVAR MÜDÜRLÜĞÜ)	TURKEY

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The report has been authorised for publication by Ursula Vincent, Head of Unit F.5.

Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised the proficiency test (PT) "GMFF-22/02" 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 dry all-in mix for preparing multigrain bread spiked with flour of maize event 59122 (Unique Identifier DAS-59122-7). Test item T2 was composed of ground soybean seeds spiked with ground seeds of GM soybean event CV127 (Unique Identifier BPS-CV127-9). The EURL GMFF evaluated the homogeneity and stability of the test items and derived the assigned values from independent measurement datasets obtained in the EURL GMFF laboratories. The assigned values (with expanded measurement uncertainty [k=2]) were 1.75 ± 0.25 m/m % for 59122 and 1.18 ± 0.17 m/m % for CV127.

Sixty-three laboratories participated to the PT round, consisting of 48 NRLs from 24 EU Member States, 11 EU OCLs and 4 OCLs from EU-neighbouring countries.

The qualitative identification of any GM event(s) present in the test items was evaluated. All but one of the 63 laboratories tested T1 for the presence of GMOs and 56 laboratories reported the presence of the 59122 maize event (the remaining 6 laboratories reported not to have tested for this event). For T2, the presence of the CV127 soybean event was indicated in the instructions and all laboratories that analysed the test item or the GM event (i.e. 60 out of 63) also detected it.

The quantitative results reported for the GM event in T1 and T2 were evaluated using z and zeta (ζ) scores, in accordance with ISO 13528:2015 [3]. The relative standard deviation for proficiency assessment (σ_{pt}) for both GM events was set to 25 % of the respective assigned values, based on the experience acquired in previous PT rounds.

Among the 63 participants having registered for this PT round, 11 and 10 did not report any quantitative result for T1 and T2, respectively, while 2 reported truncated values (greater than) for CV127. The vast majority (over 90 %) of the other laboratories proved their satisfactory performance (expressed as z score) for the analysis of 59122 maize in multigrain bread mix and CV127 soybean in soybean flour. One NRL, however, reported that the content of 59122 maize was below their LOQ (0.1 %). All participants (except two OCLs) reported their expanded measurement uncertainty and coverage factor associated with their respective measurement values. More than 86 % of the laboratories that had quantified the GM events properly assessed the compliance of the two test items investigated. The few mistakes regarding compliance evaluation concerned the inappropriate consideration of the measurement uncertainty when comparing the measured value against the labelling threshold or making reference to Regulation (EU) No 619/2011, which does not apply for these GM events.

The evaluation of this PT round confirms that most NRLs and OCLs are able to monitor and quantify mass fractions of GMOs in food and feed samples in the frame 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 **determination of the mass fractions of GM maize event 59122 in multigrain bread mix and GM soybean event CV127 in soybean flour,** 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 2022, 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. A dry mix for preparing multigrain bread (food test item T1) was selected to resemble food products analysed by control laboratories in the EU. The second sample (feed test item T2) consisted of ground whole soybean flour spiked with ground seed powder of CV127 soybean.

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.

The PT was mandatory for the NRL/625, recommended for NRL/120, and open to OCLs (under certain conditions). Participants were also asked to provide a compliance statement for each test item in relation to the applicable EU Regulations (EC) No 1829/2003 [5] and (EU) No 619/2011 [6].

This PT, organised in line with ISO/IEC 17043:2010 [2], is identified as "**GMFF-22/02**".

3 Set up of the exercise

3.1 Quality assurance

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

- ISO/IEC 17025:2017 (certificate number: BELAC 268-TEST, flexible scope for genetically modified content in % (m/m) and % (cp/cp) in food and feed); and
- ISO/IEC 17043:2010 (certificate number: BELAC 268-PT, proficiency test provider)

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

The organisation of the GMFF-22/02 exercise was announced by invitation letters to NRLs and some non-EU OCLs on October 4, 2022 (Annex 1). The registration deadline was set to October 14, 2022. Samples were sent to participants on November 8, 2022. The deadline for reporting of results was set to December 23, 2022. This deadline was extended to the end of December, 2022 upon request of some participants.

3.4 Distribution

Each participant received:

- One bottle of test item T1 (multigrain bread mix), containing approx. 5 g of dry powder;
- One bottle of test item T2 (soybean flour), containing approx. 5 g of dry powder;
- A general "Test item accompanying letter" (Annex 2).

Samples were dispatched at room temperature.

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 lab code to be used by every participant when submitting the results obtained.

The test items were described as two ground test materials, "*derived from imported samples that are not declared as containing GM material"*. The testing laboratories were requested to screen for the presence of GMOs and assess the compliance of the samples with the applicable GMO legislation (assuming that all GMO presence would be adventitious or technically unavoidable).

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

Participants were requested to perform the following analyses:

Test Item 1 – Multigrain bread mix (food):

- Verify the presence of GM maize in this sample;
- Quantify the GM event(s) identified and assess compliance of the sample.

Test Item 2 – Soybean flour (for feed):

- Verify the presence of soybean event CV127 in this sample;
- Quantify the CV127 content and assess compliance of the sample.

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 %. Since the homogeneity study was performed with 200 mg sample intake for T1 and T2, the recommended minimum sample intake was set to this amount.

When reporting the results, participants were instructed to select the appropriate setting "absent", "present", "not tested" (for qualitative tests), or "m/m %" (when entering a quantitative value), and 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 measurements and the laboratories, including on the identification (qualitative analysis) of the GM event(s) in the test items.

4 Test item

4.1 Preparation

Test item T1 was prepared from commercial flour for multigrain bread with BIO label. The flour contained maize as ingredient (confirmed by qPCR) and traces of soybean, rapeseed and rice were found using pre-spotted plates [7, 8], but no GM events were detected, which is consistent with its BIO label. Total maize content was estimated as < 5 %.

The flour was mixed with pure flour of maize 59122 previously used for producing the CRM series ERM-BF424. The resulting mixture was then homogenised in a 3-dimensional Dynamix CM200 for 1 h. Further details on the processing can be found in Table 1.

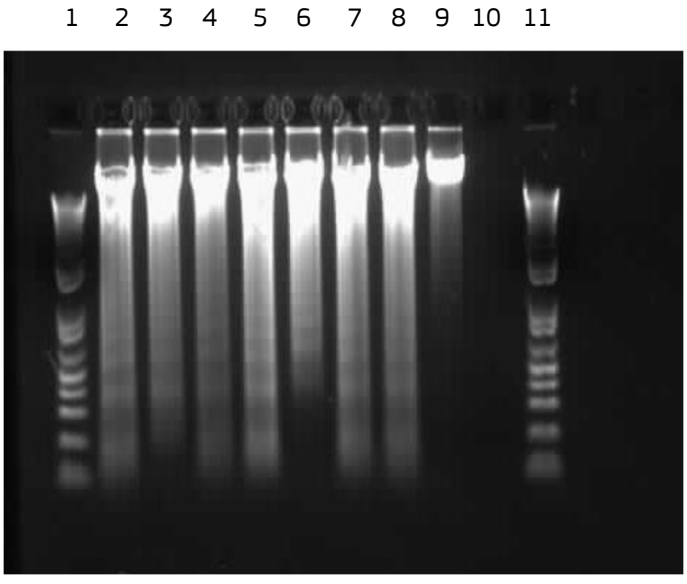
Table 1. Characteristics of the base materials used for the preparation of T1

Characteristic	Multigrain bread mix	59122 maize
Type of base material	Crude powder	Fine flour
Origin	Local grocery AVEVE (BE)	100 % 59122 flour used to prepare the ERM-BF424 series
Grinding equipment	Cryo-grinding vibrating mill	/
Mixing equipment	Dynamix CM200	
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	4.52 ± 0.09	2.36 ± 0.30
Particle diameter in µm, mean ± *U*[1] (*k*=2, *n*=3)	61.1 ± 10.8	99.9 ± 17.7
Mass used to prepare T1 (g) – STEP 1	140.00	2.52
Mass used to prepare T1 (g) – STEP 2	557.48	142.52 g of step 1

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

The final T1 flour had an average particle size (average equivalent sphere diameter of the X_{50} size class on the cumulative volume distribution curve) of 61.6 ± 10.9 µm (*k*=2, *n*=3) and a water content of 4.52 ± 0.64 g/100 g (*k*=2, *n*=3). The amount and the quality of the DNA extracted from the T1 material using a CTAB method with Genomic-tip20 purification were verified by UV spectrometry, fluorometry, and gel electrophoresis (Figure 1). A selection of DNA extracts were tested for inhibition with the *hmg* target using serial dilutions and passed the evaluation criteria (slope and ΔCq).

Figure 1. Agarose gel electrophoresis of genomic DNA extracted from the T1 material (lanes 2-9, lane 10 is an extraction blank). The molecular marker in the first and last lane is a 1 kb Plus DNA ladder (Invitrogen, USA).



The T1 mixture was manually filled using a vibrating feeder and a balance into 20 mL glass vials (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 120 vials were produced.

Test item T2 consisted of ground organic soybean, spiked with seed powder of GM soybean event CV127, received from BASF for this purpose. The CV127 powder was first cryoground, then mixed with the non-GM soybean flour, and filled in 5 g portions into 20 ml vials, closed under argon. A total of 120 vials were produced. Further details on the processing can be found in Table 2.

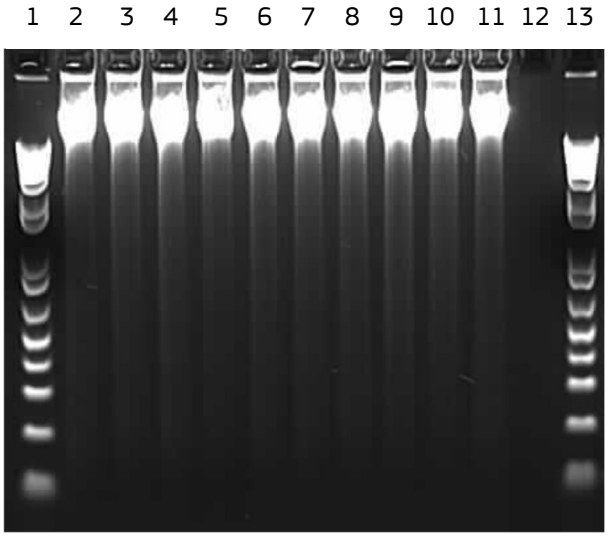
The amount and the quality of the DNA extracted from the T2 material using a CTAB method with Genomic-tip20 purification were verified by UV spectrometry, fluorometry and gel electrophoresis (Figure 2). A selection of DNA extracts were tested for inhibition with the *le1* target using serial dilutions and passed the evaluation criteria (slope and ΔCq).

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

Characteristic	Non-GM Soybean	GM Soybean CV127
Type of base material	Seeds	Coarse flour
Origin	Pit & Pit (BE) Bio–Organic Soybeans	100 % CV127 soybean
Grinding equipment	Cryo-grinding vibrating mill	Cryo-grinding vibrating mill
Mixing equipment	DynaMIX CM-200	
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	3.11 ± 0.20	6.02 ± 0.39
Particle diameter in µm, mean ± *U*[1] (*k*=2, *n*=3)	104.3 ± 18.4	89.8 ± 15.9
Mass used to prepare T2 (g) – STEP 1	103.20	8.94
Mass used to prepare T2 (g) – STEP 2	670.56	112.14 g of step 1

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

Figure 2. Agarose gel electrophoresis of genomic DNA extracted from the T2 material (lanes 2-11, lane 12 is an extraction blank). The molecular marker in the first and last lane is a 1 kb Plus DNA ladder (Invitrogen, USA).



4.2 Homogeneity and stability

Measurements for the homogeneity and stability studies, using the corresponding event-specific detection methods (with *hmg* and *Le1* as taxon-specific reference target for T1 and T2, respectively), and the statistical treatment of the data were performed by the EURL GMFF.

The assessment of **homogeneity** was performed after the processing and bottling of the test items and before distribution to the participants.

For T1, six bottles were randomly selected and 5 independent replicates per bottle were used for DNA extraction (CTAB/tip20) and qPCR analysis (for the data: see Annex 4.1). Results were evaluated according to ISO 13528:2015 [3]. The contribution from homogeneity (u_{hom}) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated using single-factor ANOVA. The T1 material proved to be adequately homogeneous for the GM event (Annex 4.1).

For T2, ten bottles were randomly selected and 5 independent replicates per bottle were used for DNA extraction (CTAB/tip20) and qPCR analysis (for the data: see Annex 4.1). We used this extended homogeneity study to assess the design of these studies and whether fewer bottles/replicates could be used in the future for the same purpose, i.e. evaluating the homogeneity of the test items. Results were evaluated according to ISO 13528:2015 [3]. The contribution from homogeneity (u_{hom}) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated using single-factor ANOVA. The T2 material proved to be adequately homogeneous for the GM event (Annex 4.1).

The stability during 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 +40 °C. The measurements by qPCR were performed under repeatability conditions. The results revealed no significant influence of storage at +40 °C on the stability of either test item (compared to storage at a reference temperature of -18 °C). The materials were therefore dispatched at room temperature.

The **long-term stability** of the test items during the extended period covered by the PT round was also tested using qPCR, analysing the GM content in bottles (N=2, n=3) stored at the normal storage temperature of +4 °C, which 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 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 (loss/increase 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. The uncertainty contribution to the assigned value due to instability was set to zero (u_{stab}=0) for the investigated measurands [3].

5 Assigned values and corresponding uncertainties

5.1 Assigned values

The assigned values (x_{pt}) for the mass fraction of the 59122 event in T1 and the CV127 event in T2 were derived from measurement results obtained by qPCR and ddPCR, applied to DNA extracted by the CTAB or CTAB/tip20 methods (Table 3). The nominal fractions of 59122 in T1 and CV127 in T2 were 1.8 and 1.2 m/m %, respectively, hence close to the averages of the measured values (=assigned values).

Table 3. Assigned values (x_{pt}) and standard deviation for the proficiency assessment (σ_{pt}) for T1 and T2 (in m/m %)

Test item	GM event	PCR method	Measured average per dataset ± *U* (*k*=2)	x_{pt}	u_{char}	u_{hom}	$u(x_{pt})$	σ_{pt}	$u(x_{pt})/\sigma_{pt}$
T1	59122	qPCR (*n*=30) [1]	1.72 ± 0.61	**1.75**	0.10	0.08	**0.12**	**0.44**	0.28
		qPCR (*n*=35) [1]	1.57 ± 0.47						
		qPCR (*n*=15) [2]	1.66 ± 0.38						
		qPCR (*n*=6) [1]	1.67 ± 0.58						
		ddPCR (*n*=14) [1]	2.12 ± 0.75						
T2	CV127	qPCR (*n*=35) [1]	1.17± 0.26	**1.18**	0.08	0.03	**0.08**	**0.30**	0.28
		qPCR (*n*=15) [1]	1.13 ± 0.28						
		qPCR (*n*=15)[1]	1.03 ± 0.14						
		ddPCR (*n*=15) [1]	1.40 ± 0.33						

[1] Laboratory 1 used a CTAB/genomic-tip20 DNA extraction method
[2] Laboratory 2 used a CTAB DNA extraction method without genomic-tip20 purification

5.2 Associated measurement uncertainties

The associated standard uncertainties of the assigned values ($u(x_{pt})$) were 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 13528:2015 [3]:

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

The uncertainty u_{char} is estimated according to the recommendations of ISO 13528:2015 [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

Only validated methods were used during the characterisation study. All values are traceable to the SI unit as a result of the use of a common CRM with certified values traceable to the SI unit. This traceability to the same reference is also confirmed by the agreement of results within their respective uncertainties. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

5.4 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviation for PT assessment (σ_{pt}) was set to 25 % of the respective assigned values, based on the experience acquired in previous PT rounds (Table 3).

6 Scores and evaluation criteria

Laboratory competence for the (qualitative) identification of a GM event in a test item was evaluated. This information had to be selected from a drop down menu (absent [default], present, not tested or m/m %) when reporting the results through the JRC electronic platform MILC, as indicated in the instructions letter. It is expected that all laboratories who have the sample matrix and the GM event within their scope of analysis should be able to identify any GM event present in the test items.

For both T1 and T2, the individual laboratory performance for the determination of the GM content was expressed in terms of *z* and ζ scores according to ISO 13528:2015 [3]:

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

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

where: x_i is the measurement result reported by a participant;

$u(x_i)$ is the standard measurement uncertainty reported by a participant;

x_{pt} is the assigned value;

$u(x_{pt})$ is the standard measurement uncertainty of the assigned value;

σ_{pt} is the standard deviation for proficiency test assessment.

The interpretation of the *z* and ζ performance scores is done according to ISO 13528:2015 [3]:

|score| ≤ 2	satisfactory performance	(green in Annex 5)
2 < |score| < 3	questionable performance	(yellow in Annex 5)
|score| ≥ 3	unsatisfactory performance	(red in Annex 5)

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 13528:2015 §7.6) or, if applicable, by formulation (ISO 13528:2015 §7.3) or than the certified measurement uncertainty associated with a certified reference material property value (ISO 13528:2015 §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-eight NRLs from 24 EU Member States (excluding Estonia, Malta and Ireland; Estonia and Ireland designated respectively Bior in Latvia and Wageningen Food Safety Research in The Netherlands as NRL for GMO analysis) and 15 OCLs registered to this PT round (Table 4). NRLs performing official controls under Regulation (EU 2017/625 (NRL/625) represented 56 % of all participants.

Table 4. Overview of participants to GMFF-22/02 by country and category

Country	Participants	NRL/625	NRL/120	OCL (not NRL)
Austria	2	2		
Belgium	3	3		
Bulgaria	2	2		
Croatia	2	2		
Cyprus	1	1		
Czech Republic	1	1		
Denmark	1	1		
Estonia	0	0		
Finland	2	1	1	
France	3	3		
Germany	14	1	11	2
Greece	1	1		
Hungary	2	1		1
Ireland	0	0		
Italy	5	1	1	3
Latvia	1	1		
Lithuania	1	1		
Luxembourg	1	1		
Malta	0	0		
Netherlands	1	1		
Poland	3	2		1
Portugal	1	1		
Romania	2	1		1
Serbia	1			1
Slovakia	2	2		
Slovenia	1	1		
Spain	6	3		3
Sweden	1	1		
Switzerland	2			2
Turkey	1			1
Total	**63**	**35**	**13**	**15**

7.2 Qualitative results

All but a few laboratories reported qualitative results: 2 laboratories (L46 and L47) reported only the results of screening tests for T1 and T2. Two NRLs indicated in the questionnaire that they did not test either T1 or T2 (matrix out of scope). Another OCL (L47) indicated not to have tested T2. In addition, respectively 6 and 1 other laboratories indicated in the MILC reporting tool that the GM event in respectively T1 or T2 was not tested.

The qualitative results are summarised in Table 5, while the individual laboratory results are presented in Annex 5.

The first step in GMO analysis of routine samples often 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**, containing 59122 maize, all 56 laboratories that had tested the GM event in the sample detected it. The event-specific identification often followed the application of a varying combination of screening tests, e.g. p35S (present), tNOS (absent) and PAT (present).

For **T2**, the presence of CV127 was communicated in the instructions and the only task was to quantify its content. A total of 60 laboratories (95 %) detected the CV127 event, while the remaining 3 laboratories did not test T2 or the GM event.

It is concluded that all laboratories that tested the sample and corresponding GM event demonstrated their capacity to identify the correct GM event in both test matrices.

Table 5. Qualitative identification of the GM events in T1 and T2 expressed as number of laboratories

Test item and/or GM event tested?	Outcome	Detailed outcome	59122 in T1	CV127 in T2
Tested	Detected (D)	Only presence reported	4	5
		Quantitative result reported	52[a]	53[b]
		Truncated value reported	0	2
	Not detected (ND)	Absence reported	0	0
Not tested (NT)		Test item not tested	1	2
		GM event not tested	6	1
Total			63	63

[a] Three laboratories reported both a qPCR and dPCR result for this event
[b] Four laboratories reported both a qPCR and dPCR result for this event

7.3 Quantitative results

7.3.1 Performance

A total of 52 or 53 (out of 63) laboratories reported quantitative results for 59122 or CV127, respectively. One laboratory (L39) reported (in the questionnaire) that the content of 59122 maize in T1 was below the LOQ of the method (0.1 %); this laboratory will be contacted for further root-cause analysis investigations.

The majority of participants applied real-time PCR, while 7 (59122) and 10 (CV127) laboratories reported digital PCR results, which is more than in previous PT rounds. Four laboratories reported quantitative results obtained by both qPCR and dPCR for CV127 (1 laboratory) or for both GM events (3 laboratories). The option to register twice to the PT round (for reporting qPCR and dPCR results) was provided to the participants (they received a labcode with extension “a” for qPCR and “b” for dPCR).

Laboratory performance for quantification of the GM events in T1 and T2 was expressed in terms of z and ζ scores. Annex 5 presents the reported results as tables and graphs for each measurand. Satisfactory performance is highlighted in green, questionable in yellow, unsatisfactory in red. Cells were left uncoloured when the outcome could not be evaluated. The corresponding Kernel density plots have been obtained using the software available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [12].

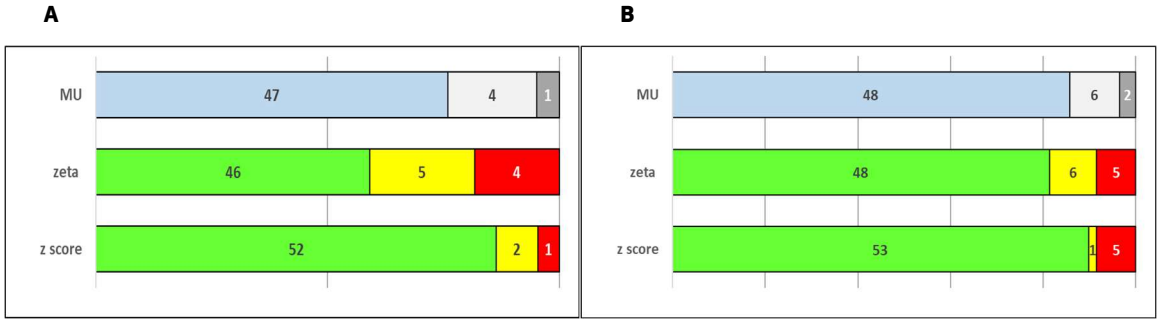
Figure 2 summarises the performance scores obtained. A total of 55 and 59 results were scored for T1 and T2, respectively (including the double results reported by 3 or 4 laboratories). An overall satisfactory performance of 95 % (59122) and 90 % (CV127) was obtained. One and five unsatisfactory results were obtained for 59122 and CV127, respectively. The unsatisfactory z score for T1 was an underestimation of the 59122 content, while all 5 unsatisfactory z scores for T2 were overestimations of the GM content.

All the laboratories that had used **digital PCR** obtained satisfactory performance (z) scores varying between -1.8 and 1.2 for T1, and between -0.9 and 1.7 for T2. The z scores of the laboratories that had reported results for qPCR and dPCR were generally in agreement, and in 3 cases closer to zero with qPCR, in 3 cases with dPCR and in one case the z scores were equal.

Figure 3. Overview of laboratory performance according to z and ζ scores, for the content of the event 59122 maize in T1 (A) and CV127 soybean in T2 (B).

Satisfactory, questionable and unsatisfactory performance scores are indicated in green, yellow and red, respectively. Corresponding numbers of laboratories 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 Truncated values

Two truncated values were reported for CV127 in T2, both of the type "more than x" (> 0.002 and > 0.1). While these values could not be included as such in the data evaluation, they were considered plausible, because they were below the $x_{pt} - U(x_{pt})$ threshold. Hence, the two GM events were correctly identified, but not quantified.

7.3.3 Measurement uncertainties

All laboratories having reported quantitative results, except L05 and L51 (OCL), provided expanded measurement uncertainties and coverage factors for both measurands (Annex 5). The missing uncertainties of these two laboratories was shown as "not provided (np)" in the tables in Annex 5.

Most of the laboratories (90 % and 86 % for 59122 and CV127, respectively) reported a realistic measurement uncertainty (Case "a" in Figure 2).

7.3.4 Compliance statement

Regulation (EC) No 1829/2003 [5] has established a threshold for labelling of food and feed products containing (adventitious or technically unavoidable) GM material that is authorised in the EU (0.9 %). Furthermore, Regulation (EU) No 619/2011 [6] has introduced a minimum performance limit (0.1 m/m %) for detecting the accidental presence, in feed, of GM material with a pending or expired authorisation status. Compliance with these values is verified by the Member States of the European Union during the 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. Participants were requested to choose among five compliance statements:

CNL Compliant because no labelling required (authorised GMO mass fraction < 0.9 m/m %, if adventitious or technically unavoidable);

C<LLP Compliant because GMO falling under Regulation 619/2011 was present at < 0.1 m/m % (assuming it was adventitious or technically unavoidable);

NCL Not compliant because the product should have been labelled (authorised GMO mass fraction > 0.9 m/m %);

NC>LLP Not compliant because the product contains GMOs falling under Regulation 619/2011 at a mass fraction above 0.1 m/m %;

CNC Cannot conclude.

It is important to understand that Regulation 1829/2003 and 619/2011 are mutually exclusive, i.e. a product is either:

i). compliant to Regulation (EC) 1829/2003, when the GM event is authorised and present at a level ≤ 0.9 m/m %,

or

ii). compliant to Regulation (EU) 619/2011, when the authorisation is pending or has expired, the event is included in the EU GM register related to this Regulation and it is present, in feed, at a level ≤ 0.1 m/m %.

A total of 122 compliance statements, provided for T1 and T2 samples by all but two laboratories (L01, L47), were evaluated,. Most laboratories provided a justification for their choice among the 5 compliance options. The option selected and the justification provided were evaluated (summarised in Tables 6 and 7). Although some testing laboratories do not usually provide such statements to their Competent Authorities when reporting their results, all laboratories should be aware of the labelling rules in the EU and should be able to properly interpret their results.

The 59122 event present in **T1** 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) where only this Regulation applies. The following assumptions were taken into account:

- The content of 59122 measured in T1 is above the threshold;
- The lower limit of the assigned (expanded, with coverage factor 2) range for 59122 is 1.75 – 0.25 = 1.50 m/m %. Taking the number of significant figures in the legislation into account, the measured value needs to be rounded to 1.5 m/m %. Hence, labelling is required for this material and as the product is not labeled as GMO (as indicated in the instructions letter for this PT) it is not compliant (NCL). Forty-two laboratories correctly selected this statement.
- On the basis of the measurement results obtained in the laboratory it is possible that $x - U \leq 0.9$ m/m %, in which case the sample should be considered compliant to Regulation (EC) 1829/2003 because labelling is then not required (CNL). Seven laboratories correctly selected this statement.

The majority of laboratories (49 out of 51, excluding the 9 inconclusive answers) reported a correct compliance statement (Table 6). Two laboratories additionally answered either C<LLP or NC>LLP, which is incorrect as Regulation (EU) No 619/2011 does not apply to food products and no GM events were detected that are listed with pending or expired authorisation status.

Table 6. Reported compliance statements for T1 (multigrain bread mix)

Compliance Statement	Laboratory Measurement	Number of Laboratories [a]	Comment
CNL - Compliant, because no labelling required	$x \pm U \leq 0.9$ m/m %	7	
	$x \pm U > 0.9$ m/m %	0	
NCL - Not compliant, should have been labelled	$x \pm U > 0.9$ m/m %	42	
	$x \pm U \leq 0.9$ 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	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 %	1	Wrong as this Regulation does not apply
CNC - Cannot conclude / not quantified		9	
Total no. of results for which a statement was provided		60	

[a] Some participants provided more than one answer on compliance for the same sample

For **T2** a similar assessment was made. The CV127 soybean 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 % and only this Regulation applies. The following assumptions were taken into account:

- The content of CV127 measured in T2 is above the threshold (1.18 m/m %).
- The lower limit of the assigned (expanded, with coverage factor 2) range for CV127 is 1.18 – 0.17 = 1.01 m/m %. Taking the number of significant figures in the legislation into account, the measured value needs to be rounded to 1.0 m/m %. Hence, labelling is required for this material and as the product is not labeled as GMO (as indicated in the instructions letter for this PT) it is not compliant (NCL).
- Eighteen laboratories correctly selected this statement, however, 3 laboratories did not take the rounding of the final result into account (e.g. 0.94 was considered > 0.9). Four other laboratories that had selected this option did not properly take the measurement uncertainty into account and should have selected CNL (Table 7).
- On the basis of the measurement results obtained in the laboratory it is possible that $x - U \leq 0.9$ m/m %, in which case the sample should be considered not compliant to Regulation (EC) 1829/2003 because labelling is then required (CNL). Twenty-eight laboratories correctly selected this statement (Table 7). One laboratory (L13) that had selected this option should rather have selected CNL because the $x - U$ was 0.97, which is > 0.9 m/m % after rounding.

The majority of laboratories (46 out of 54, excluding the 8 inconclusive answers) reported a correct compliance statement. Three laboratories answered NC>LLP, which is incorrect as Regulation (EU) No 619/2011 does not apply since no GM events that are listed with pending or expired authorisation status were detected.

Several laboratories were unsure about the compliance of the sample (hence reported CNC) because no quantification had been done, because a non-EU legislation applied (e.g. Turkey), or for another reason.

Table 7. Reported compliance statements for T2 (soybean flour)

Compliance Statement	Laboratory Measurement	Number of Laboratories [a]	Comment
CNL - Compliant, because no labelling required	$X \pm U \leq 0.9$ m/m %	28	
	$X \pm U > 0.9$ m/m %	1	$Xi - U$ was > 0.9, hence NCL
NCL - Not compliant, should have been labelled	$X \pm U > 0.9$ m/m %	18	
	$X \pm U \leq 0.9$ m/m %	4	$Xi - U$ was ≤ 0.9, hence CNL
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 %	3	Wrong as this Regulation does not apply
CNC - Cannot conclude / not quantified		8	
Total no. of results for which a statement was provided		60	

[a] Some participants provided more than one answer on compliance for the same sample

7.4 Questionnaire

The questionnaire was answered by all but one participant (L01) and gave valuable information about the (62) laboratories, their way of working and their analytical approaches. Detailed information is available in Annex 6, which summarises all experimental details and comments provided by the participants. Note that two laboratories reported answers applicable to both their qPCR and dPCR results (therefore the total number of answers provided equals to 64 when no double answers are accepted).

The majority of participants (64 % for T1 and 58 % for T2) reported that their laboratory was accredited in accordance with **ISO/IEC 17025** for the methods used in the PT round, but other respondents have only accreditation for some of the methods used ("partially" accredited; 22-23 %) or no accreditation (13-19 %). The laboratories that used dPCR are mostly not accredited for the method applied, except 3 laboratories for 59122 in T1, and 2 for CV127 in T2.

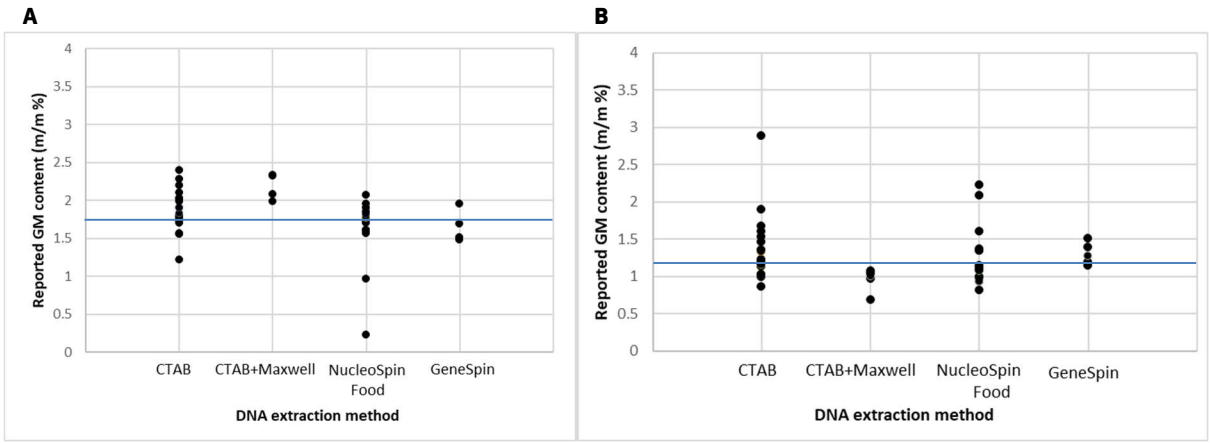
Most laboratories (57) used **screening methods** for T1 to limit the number of GMOs to test with event-specific methods. The most common screening markers were p35S, tNOS, PAT and *bar*. Also CTP2-CP4-EPSPS was often used as screening target. The screening results were not always consistent between laboratories, e.g. 49 laboratories reported the absence of tNOS, but 4 found it present, 21 found CTP2-CP4-EPSPS "absent", but 6 reported "present", etc. Screening methods were also used by a number of laboratories for T2 to verify the presence of the CV127, as requested in the instructions.

Further details reported by the participating laboratories can be found in Annex 6.

Of particular interest is to verify if there was an **effect of the DNA extraction method on the GM content** reported. Different methods were used by the laboratories, mostly based on the use of (1 or 2 %) CTAB for lysis (sometimes followed by use of an automatic purification system) or using a commercial kit such as NucleoSpin Food or GeneSpin. Comparison of the reported results against the DNA extraction method used did not reveal a correlation (Figure 3):

- For T1, the results varied between 1.22 and 2.4 m/m % for CTAB (*N*=18), between 0.97 and 2.07 m/m % for NSF (*N*=14) (with one additional value at 0.23 m/m % that was scored unsatisfactory) and between 1.48 and 1.96 m/m % for the GeneSpin kit (*N*=4). All 4 values reported for "CTAB+Maxwell RSC PureFood GMO and authentication kit" were at the high end of the mass fractions (between 1.99 and 2.33 m/m %), but the low number of observations does not allow to make conclusions.
- For T2, the results varied between 0.86 and 1.9 m/m % for CTAB (*N*=16, with an additional value at 2.89 m/m % scored unsatisfactory), between 0.82 and 1.61 m/m % for NucleoSpin Food (*N*=13, with additional values at 2.09 and 2.23 m/m % scored unsatisfactory) and between 1.15 and 1.51 m/m % for the GeneSpin kit (*N*=5). All 5 values reported for "CTAB+Maxwell RSC PureFood GMO and authentication kit" were at the low end (between 0.69 and 1.07 m/m %), but the low number of observations does not allow to make conclusions.
- Also for the other DNA extraction methods used, with fewer reported results, no effect of the extraction method on the reported result was observed. The results that were scored as unsatisfactory or questionable were obtained on DNA extracted by 2 % CTAB (2), NucleoSpin Food (2) or NucleoSpin Plant (1), while one laboratory did not report the extraction method used.

Figure 4. Effect of DNA extraction method used on reported GM quantity for T1 (A) and T2 (B). Horizontal line: assigned value.



8 Conclusions

The proficiency test GMFF-22/02 was organised to assess the analytical capabilities of EU NRLs and OCLs to analyse a food material (T1) and a feed material (T2) and to determine the content of 59122 maize and CV127 in these test items.

The vast majority of participants correctly identified the spiked GM events in T1 and T2, while 83-84 % of laboratories quantified these GM events. The overall performance of the participants for the determination of the content of both GM events in T1 and T2 was satisfactory (95 % for 59122 in T1, 90 % for CV127 in T2).

The compliance statements provided by most of the laboratories were considered in line with their reported results for T1 and T2.

It shows that the 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].

References

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

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List of abbreviations and symbols

bp	Base pairs
(d)dPCR	(Droplet) 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
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 Testing
qPCR	Quantitative (real-time) Polymerase Chain Reaction
k	Coverage factor
σ_{pt}	Standard deviation for proficiency test assessment
$u(x_i)$	Standard measurement uncertainty reported by participant "i"
$u(x_{pt})$	Standard uncertainty of the assigned value
u_{char}	(Standard) uncertainty contribution due to characterisation
u_{hom}	(Standard) uncertainty contribution due to inhomogeneity
u_{stab}	(Standard) uncertainty contribution due to instability
$U(x_i)$	Expanded uncertainty reported by participant "i" with the coverage factor k
$U(x_{pt})$	Expanded uncertainty of the assigned value with the coverage factor k
x_i	Mean value reported by participant "i"
x_{pt}	Assigned value
z (or z')	z (or z') score
ζ	zeta score

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Annexes

Annex 1. Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory for GM Food & Feed

Geel, 4 October 2022
JRC.F.5/UV/wb/mt/ARES(2022) 22-077

FOR THE ATTENTION OF THE
NATIONAL REFERENCE LABORATORIES (NRLS) FOR GMOS
UNDER REGULATIONS (EU) 2017/625 AND (EU) NO 120/2014

Subject: Invitation to participate to the Proficiency Testing round "GMFF-22/02"

Dear National Reference Laboratory representative,

On behalf of the EURL for GM Food and Feed (EURL GMFF), we would like to invite you to participate to the proficiency test (PT) "**Determination of GM maize in multigrain bread mix (T1) and GM soybean in soybean powder (T2)**". You will receive two ground test materials. You are requested to check for the presence of GM maize (T1) or GM soybean (T2), identify and quantify the GM event(s), and assess the compliance of the samples 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=2841.

Once you have submitted your registration electronically, you will have to:

- Print your registration form, as indicated on screen
- Sign it, date it and send it to us by e-mail (JRC-EURL-GMFF-CT@ec.europa.eu)

Please register by Friday 14 October 2022.

The test items will be shipped on 8 November 2022.

The deadline for submission of the results is Friday 23 December 2022.

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, the lab codes of the NRLs that have been designated in line with Regulation (EU) 2017/625 will be disclosed to DG SANTE, upon request, for (long-term) performance assessment. Lab codes of appointed official laboratories may be disclosed to their NRL upon request.

This invitation is only sent to the NRLs. You may distribute this letter to any official laboratory within your network of official control laboratories for which you deem its participation as relevant considering all or any of the requested tasks. These laboratories will have to register for this PT using the registration details provided in this letter.

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

Kind regards,

/signed electronically in Ares/ */signed electronically in Ares/*

Dr. Ursula Vincent, Head of Unit
Dr. Wim Broothaerts, PT Coordinator

Annex 2. Test item accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory for GM Food & Feed

Geel, 8 November 2022

Subject: **GMFF-22/02, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* multigrain bread mix and soybean powder**

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, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory for GM Food & Feed

Geel, 8 November 2022
JRC.F.5/WB/mt ARES(2022) 22-090

«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/GMFF2202
Password	GMFF2202

Subject: **Instructions for GMFF-22/02, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* multigrain bread mix and soybean flour**

Dear Dr «Surname»,

Thank you for participating to GMFF-22/02. 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.**

The two ground test materials are "*derived from imported samples that are 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 (assuming that all GMO presence would be adventitious or technically unavoidable).

Tasks
Test Item 1 – Multigrain bread mix (food) (5 g dry weight):
- Verify the presence of GM maize in this sample;
- Quantify the GM event(s) identified and assess compliance of the sample.

Test Item 2 - Soybean flour (for feed) (5 g dry weight):
- Verify the presence of soybean event CV127;
- Quantify the CV127 content and assess compliance of the sample.

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 "absent" for all GM events; please change this into m/m % if reporting a quantitative result, or to "present" or "not tested" 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;
- 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”).

Don't 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 **print the completed report form, sign it 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/GMFF2202, 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 30 December 2022. 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 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,

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

Annex 4. Homogeneity and stability results

4.1 Homogeneity

Homogeneity of 59122 maize in T1 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
8	1.43	1.58	1.49	1.80	1.41
88	1.42	1.50	1.30	1.84	1.81
10	1.63	1.65	1.61	1.83	2.49
11	2.02	1.88	1.11	1.98	1.57
60	1.32	1.56	1.66	1.71	1.61
61	1.95	1.75	1.33	1.60	2.24
Mean	1.67				
s_x	0.09				
s_w	0.32				
S_s	0				
u^*	0.08				
σ_{pt}	0.44				
$0.3 * \sigma_{pt}$	0.13				
$S_s \leq 0.3^* \sigma_{pt}$	YES				
Assessment	Passed				

Homogeneity of CV127 soybean in T2 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
17	1.09	0.99	1.11	1.03	1.13
22	1.12	1.15	1.02	1.13	1.14
34	1.26	1	1.13	1.34	1.21
39	1.1	1.52	1.17	1.15	1.06
50	1.2	1.16	1.22	1	1.05
59	1.01	1.08	1.16	1.12	1.21
73	1.33	1.06	1.16	1.21	1.17
77	1.13	1.09	1.3	1.17	1.59
87	1.38	1.04	1.3	1.1	1.16
94	1.23	1.15	1.41	1.06	1.28
Mean	1.17				
s_x	0.06				
s_w	0.13				
S_s	0.02				
u^*	0.03				
σ_{pt}	0.30				
$0.3 * \sigma_{pt}$	0.10				
$S_s \leq 0.3^* \sigma_{pt}$	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 13528:2015 § B.5 [3].

Stability 59122 maize in T1 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	8	1.43	1.58	1.49	1.58
	88	1.3	1.84	1.81	
30	29	2.02	1.59	1.44	1.67
	98	1.53	1.48	1.95	

Slope ± 2 $SE_{(slope)}$ = 0.0031 ± 2 * 0.0045

Stability: passed

Stability CV127 soybean in T2 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	18	1.07	1.18	0.93	1.09
	97	1.25	1.06	1.07	
30	6	0.98	1.09	0.97	1.03
	101	0.99	1.03	1.13	

Slope ± 2 $SE_{(slope)}$ = -0.0021 ± 2 * 0.0017

Stability: passed

Annex 5. Results and laboratory performance

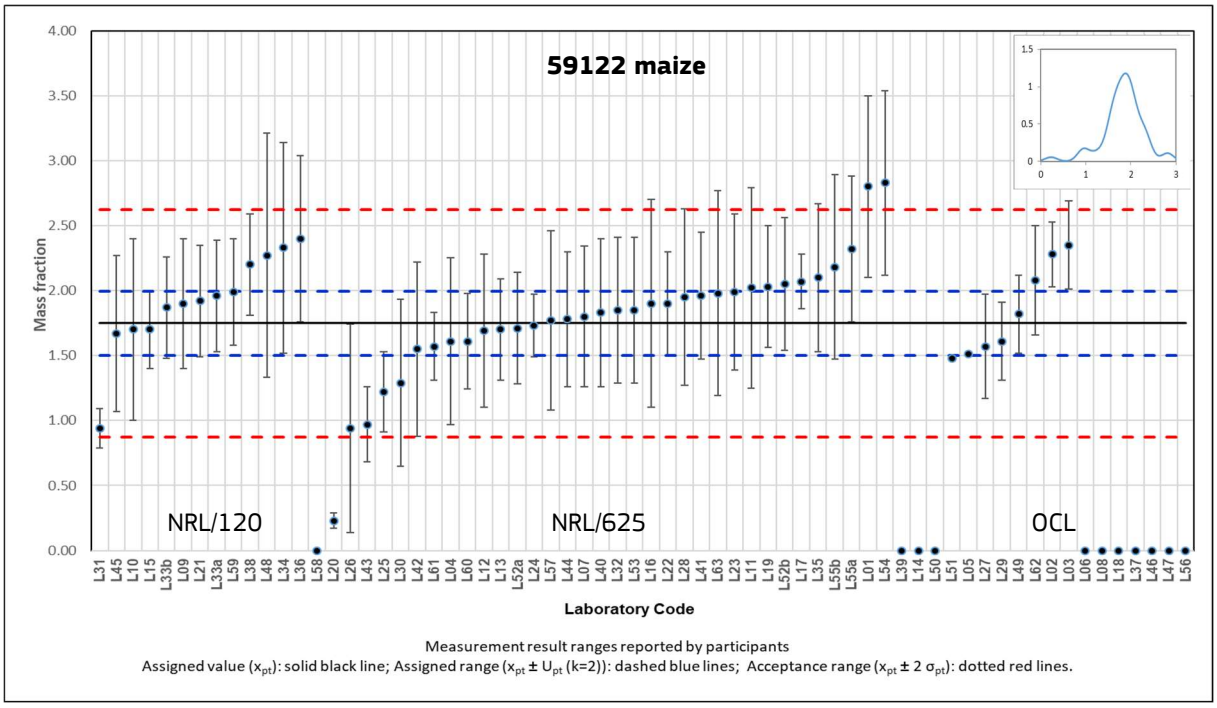
59122 maize in T1

- ID = GM event identification (D = detected, ND = not detected, NT = not tested)
- The PT coordinator set k = 1.73 when no coverage factor (k) was reported
- Performance scores (z and ζ): satisfactory, questionable, unsatisfactory
- 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: $u_{rel}(x_i) > \sigma_{pt,\%}$; or NP (not provided)
- Compliance (Compl.) statements (in red if incorrect):
  CNL: compliant, no labelling required; C<LLP: compliant because <0.1 m/m % under Reg. 619/2011; NCL: not compliant because should have been labelled; NC>LLP: not compliant because >0.1 m/m % under Reg. 619/2011; CNC: cannot conclude; "--" no answer.

Evaluation parameters: x_{pt} = 1.75 ; $u(x_{pt})$ = 0.12 ; σ_{pt} = 0.44 (all values in m/m %)

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L01	NRL/625	D	2.8	0.7	2	rt-PCR	2.4	2.8	a	--
L02	OCL	D	2.28	0.25	2	dPCR	1.2	3.0	b	NCL
L03	OCL	D	2.35	0.34	2	rt-PCR	1.4	2.9	a	NCL
L04	NRL/625	D	1.61	0.64	2	rt-PCR	-0.3	-0.4	a	NCL
L05	OCL	D	1.51			rt-PCR	-0.5	-1.9	NP	CNC
L06	OCL	D				rt-PCR				CNC
L07	NRL/625	D	1.8	0.54	2	rt-PCR	0.1	0.2	a	NCL
L08	OCL	NT								CNC
L09	NRL/120	D	1.9	0.5	2.36		0.3	0.6	a	NCL
L10	NRL/120	D	1.7	0.7	4.3	rt-PCR	-0.1	-0.2	a	NCL
L11	NRL/625	D	2.02	0.77	2	rt-PCR	0.6	0.7	a	NCL
L12	NRL/625	D	1.69	0.59	2	rt-PCR	-0.1	-0.2	a	NCL
L13	NRL/625	D	1.7	0.39	2	rt-PCR	-0.1	-0.2	a	NCL
L14	NRL/625	NT								--
L15	NRL/120	D	1.7	0.3	2	rt-PCR	-0.1	-0.2	a	NCL
L16	NRL/625	D	1.9	0.8	2	rt-PCR	0.3	0.4	a	NCL, C<LLP
L17	NRL/625	D	2.07	0.21	2	rt-PCR	0.7	2.0	b	NCL
L18	OCL	D								CNC
L19	NRL/625	D	2.03	0.47	2	rt-PCR	0.6	1.1	a	NCL
L20	NRL/625	D	0.23	0.06	2	rt-PCR	-3.5	-11.9	a	CNL
L21	NRL/120	D	1.92	0.43	2	rt-PCR	0.4	0.7	a	CNC
L22	NRL/625	D	1.9	0.4	2	rt-PCR	0.3	0.6	a	NCL
L23	NRL/625	D	1.99	0.6	2	rt-PCR	0.6	0.7	a	NCL
L24	NRL/625	D	1.73	0.24	2	rt-PCR	0.0	-0.1	b	NCL
L25	NRL/625	D	1.22	0.31	2	rt-PCR	-1.2	-2.7	a	NCL
L26	NRL/625	D	0.94	0.8	2	rt-PCR	-1.8	-1.9	c	CNL
L27	OCL	D	1.57	0.4	2	rt-PCR	-0.4	-0.8	a	NCL
L28	NRL/625	D	1.95	0.68	2	rt-PCR	0.5	0.6	a	NCL
L29	OCL	D	1.61	0.3	2	dPCR	-0.3	-0.7	a	NCL
L30	NRL/625	D	1.29	0.64	2	rt-PCR	-1.0	-1.3	a	CNL
L31	NRL/120	D	0.94	0.15	2.36	dPCR	-1.8	-5.8	b	CNL
L32	NRL/625	D	1.85	0.56	2	rt-PCR	0.2	0.3	a	NCL
L33a	NRL/120	D	1.96	0.43	2	rt-PCR	0.5	0.9	a	NCL
L33b	NRL/120	D	1.87	0.39	2	dPCR	0.3	0.5	a	NCL
L34	NRL/120	D	2.33	0.81	2	dPCR	1.3	1.4	a	NCL
L35	NRL/625	D	2.1	0.57	2	rt-PCR	0.8	1.1	a	CNC
L36	NRL/120	D	2.4	0.64	2	rt-PCR	1.5	1.9	a	NCL
L37	OCL	NT								--

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L38	NRL/120	D	2.2	0.39	1	dPCR	1.0	1.1	a	NCL, NC>LLP
L39	NRL/625	D	[<LOQ]			rt-PCR				CNL
L40	NRL/625	D	1.83	0.57	2	rt-PCR	0.2	0.3	a	NCL
L41	NRL/625	D	1.96	0.49	2	rt-PCR	0.5	0.8	a	NCL
L42	NRL/625	D	1.55	0.67	2	rt-PCR	-0.5	-0.6	a	CNL
L43	NRL/625	D	0.97	0.29	2	rt-PCR	-1.8	-4.1	a	CNL
L44	NRL/625	D	1.78	0.52	2	rt-PCR	0.1	0.1	a	NCL
L45	NRL/120	D	1.67	0.6	2	rt-PCR	-0.2	-0.2	a	NCL
L46	OCL	NT								CNC
L47	OCL	NT								--
L48	NRL/120	D	2.27	0.94	2	rt-PCR	1.2	1.1	a	NCL
L49	OCL	D	1.82	0.3	2	rt-PCR	0.2	0.4	a	NCL
L50	NRL/625	NT								--
L51	OCL	D	1.48			rt-PCR	-0.6	-2.2	NP	--
L52a	NRL/625	D	1.71	0.43	2	rt-PCR	-0.1	-0.2	a	NCL
L52b	NRL/625	D	2.05	0.51	2	dPCR	0.7	1.1	a	NCL
L53	NRL/625	D	1.85	0.56	2	rt-PCR	0.2	0.3	a	NCL
L54	NRL/625	D	2.83	0.71	2	rt-PCR	2.5	2.9	a	NCL
L55a	NRL/625	D	2.32	0.56	2	rt-PCR	1.3	1.9	a	NCL
L55b	NRL/625	D	2.18	0.71	2	dPCR	1.0	1.1	a	NCL
L56	OCL	NT								CNC
L57	NRL/625	D	1.77	0.69	2	rt-PCR	0.1	0.1	a	NCL
L58	NRL/120	D				rt-PCR				CNC
L59	NRL/120	D	1.99	0.41	2.78	rt-PCR	0.6	1.3	a	NCL
L60	NRL/625	D	1.61	0.37	2	rt-PCR	-0.3	-0.6	a	NCL
L61	NRL/625	D	1.57	0.26	2	rt-PCR	-0.4	-1.0	a	NCL
L62	OCL	D	2.08	0.42	2	rt-PCR	0.8	1.36	a	NCL
L63	NRL/625	D	1.98	0.79	2	rt-PCR	0.5	0.6	a	NCL



Upper right: kernel density distribution

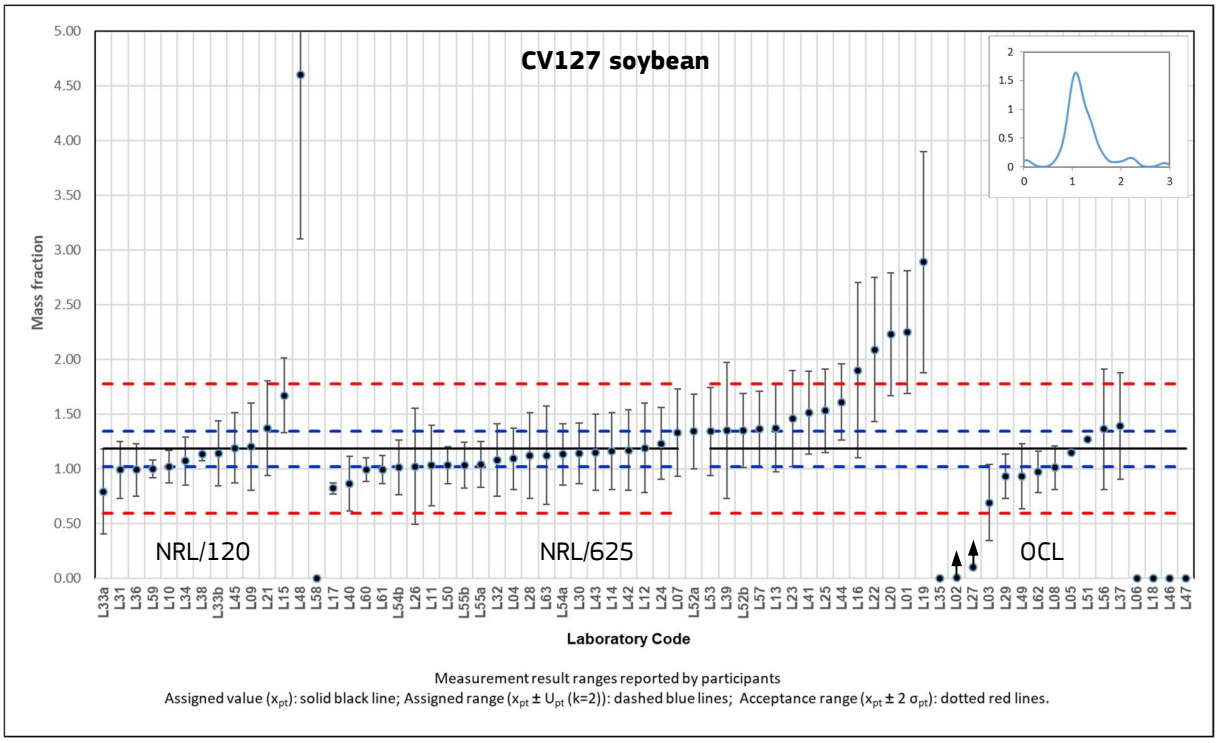
CV127 soybean in T2

- ID = GM event identification (D = detected, ND = not detected, NT = not tested)
- The PT coordinator set k = 1.73 when no coverage factor (k) was reported
- Performance scores (z and ζ): satisfactory, questionable, unsatisfactory
- 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: $u_{rel}(x_i) > \sigma_{pt,\%}$; or NP (not provided)
- Compliance (Compl.) statements (in red letters if incorrect):
  CNL: compliant, no labelling required; C<LLP: compliant because <0.1 m/m % under Reg. 619/2011; NCL: not compliant because should have been labelled; NC>LLP: not compliant because >0.1 m/m % under Reg. 619/2011; CNC: cannot conclude; "--" no answer.

Evaluation parameters: x_{pt} = 1.18 ; $u(x_{pt})$ = 0.08 ; σ_{pt} = 0.30 (all values in m/m %)

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L01	NRL/625	D	2.25	0.56	2	rt-PCR	3.6	3.7	a	--
L02	OCL	D	>0.002							CNC
L03	OCL	D	0.69	0.35	2	rt-PCR	-1.7	-2.5	c	CNL
L04	NRL/625	D	1.09	0.28	2	rt-PCR	-0.3	-0.6	a	CNL
L05	OCL	D	1.15			rt-PCR	-0.1	-0.4	NP	CNC
L06	OCL	D				rt-PCR				CNC
L07	NRL/625	D	1.33	0.4	2	rt-PCR	0.5	0.7	a	NCL
L08	OCL	D	1.01	0.2	2	rt-PCR	-0.6	-1.3	a	NCL
L09	NRL/120	D	1.2	0.4	2.45	dPCR	0.1	0.1	a	CNC
L10	NRL/120	D	1.02	0.15	3.18	rt-PCR	-0.5	-1.7	b	CNL
L11	NRL/625	D	1.03	0.37	2	rt-PCR	-0.5	-0.8	a	CNL
L12	NRL/625	D	1.19	0.41	2	rt-PCR	0.0	0.0	a	CNL
L13	NRL/625	D	1.37	0.4	2	rt-PCR	0.6	0.9	a	CNL
L14	NRL/625	D	1.16	0.35	2	rt-PCR	-0.1	-0.1	a	NCL
L15	NRL/120	D	1.67	0.34	2	dPCR	1.7	2.6	a	NCL
L16	NRL/625	D	1.9	0.8	2	rt-PCR	2.4	1.8	a	NCL
L17	NRL/625	D	0.82	0.05	2	rt-PCR	-1.2	-4.2	b	CNL
L18	OCL	D				rt-PCR				CNC
L19	NRL/625	D	2.89	1.01	2	rt-PCR	5.8	3.3	a	NCL
L20	NRL/625	D	2.23	0.56	2	rt-PCR	3.5	3.6	a	NCL
L21	NRL/120	D	1.37	0.43	2	rt-PCR	0.6	0.8	a	NCL
L22	NRL/625	D	2.09	0.66	2	rt-PCR	3.1	2.7	a	NCL
L23	NRL/625	D	1.46	0.44	2	rt-PCR	0.9	1.2	a	NCL
L24	NRL/625	D	1.23	0.33	2	rt-PCR	0.2	0.3	a	NCL
L25	NRL/625	D	1.53	0.38	2	rt-PCR	1.2	1.7	a	NCL
L26	NRL/625	D	1.02	0.53	2	rt-PCR	-0.5	-0.6	c	CNL
L27	OCL	D	>0.1			rt-PCR				CNC
L28	NRL/625	D	1.12	0.39	2	rt-PCR	-0.2	-0.3	a	CNL
L29	OCL	D	0.93	0.2	2	dPCR	-0.9	-1.9	a	NCL
L30	NRL/625	D	1.14	0.28	2	rt-PCR	-0.1	-0.3	a	CNL
L31	NRL/120	D	0.99	0.26	2.36	dPCR	-0.6	-1.4	a	CNL
L32	NRL/625	D	1.08	0.33	2	rt-PCR	-0.3	-0.6	a	CNL
L33a	NRL/120	D	0.79	0.39	2	rt-PCR	-1.3	-1.9	a	CNL
L33b	NRL/120	D	1.14	0.3	2	dPCR	-0.1	-0.2	a	CNL
L34	NRL/120	D	1.07	0.22	2	dPCR	-0.4	-0.8	a	CNL
L35	NRL/625	NT								--
L36	NRL/120	D	0.99	0.24	2	rt-PCR	-0.6	-1.3	a	CNL
L37	OCL	D	1.39	0.49	2	rt-PCR	0.7	0.8	a	CNL
L38	NRL/120	D	1.13	0.06	2	dPCR	-0.2	-0.6	b	NCL, NC>LLP
L39	NRL/625	D	1.35	0.62	2	rt-PCR	0.6	0.5	a	CNL

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L40	NRL/625	D	0.86	0.25	2	rt-PCR	-1.1	-2.2	a	CNL
L41	NRL/625	D	1.51	0.38	2	rt-PCR	1.1	1.6	a	NC>LLP
L42	NRL/625	D	1.17	0.37	2	rt-PCR	0.0	-0.1	a	CNL
L43	NRL/625	D	1.15	0.35	2	rt-PCR	-0.1	-0.2	a	CNL
L44	NRL/625	D	1.61	0.35	2	rt-PCR	1.4	2.2	a	NCL
L45	NRL/120	D	1.19	0.32	2	rt-PCR	0.0	0.0	a	CNL
L46	OCL	NT								CNC
L47	OCL	NT								--
L48	NRL/120	D	4.6	1.5	2	rt-PCR	11.6	4.5	a	NCL
L49	OCL	D	0.93	0.3	2	rt-PCR	-0.9	-1.5	a	NCL
L50	NRL/625	D	1.03	0.17	2		-0.5	-1.3	a	CNL
L51	OCL	D	1.27			rt-PCR	0.3	1.1	NP	--
L52a	NRL/625	D	1.34	0.34	2	rt-PCR	0.5	0.8	a	NCL
L52b	NRL/625	D	1.35	0.34	2	dPCR	0.6	0.9	a	NCL
L53	NRL/625	D	1.34	0.4	2	rt-PCR	0.5	0.7	a	NCL
L54a	NRL/625	D	1.13	0.28	2	rt-PCR	-0.2	-0.3	a	CNL
L54b	NRL/625	D	1.01	0.25	2	dPCR	-0.6	-1.2	a	CNL
L55a	NRL/625	D	1.04	0.21	2	rt-PCR	-0.5	-1.1	a	CNL
L55b	NRL/625	D	1.03	0.21	2	dPCR	-0.5	-1.1	a	CNL
L56	OCL	D	1.36	0.55	2	rt-PCR	0.6	0.6	a	NCL
L57	NRL/625	D	1.36	0.35	2	rt-PCR	0.6	0.9	a	NCL
L58	NRL/120	D				rt-PCR				CNC
L59	NRL/120	D	1	0.08	2.57	rt-PCR	-0.6	-2.1	b	NCL
L60	NRL/625	D	0.99	0.108	2	rt-PCR	-0.6	-2.0	b	NC>LLP
L61	NRL/625	D	0.99	0.13	2	rt-PCR	-0.6	-1.8	b	CNL
L62	OCL	D	0.97	0.19	2	rt-PCR	-0.7	-1.69	a	CNL
L63	NRL/625	D	1.12	0.45	2	rt-PCR	-0.2	-0.3	a	CNL



Upper right: kernel density distribution

Annex 6. 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.

Please select which test items were analysed by your laboratory

	T1	T2
Yes	62	62
No	2*	2*
No Answer	0	0

* Reasons mentioned: The sample matrix is out of the scope of our laboratory, the method is not validated in the laboratory, CRM or primers/probes were not available, and "other"

Are the methods used within the scope of accreditation of your laboratory under ISO/IEC 17025:2017?

	T1	Ratio	T2	Ratio
Yes	41	64.06%	37	57.81%
No	8	12.5%	12	18.75%
Partially	15	23.44%	14	21.88%
Not applicable	0	0%	1	1.56%
No Answer	0	0%	0	0%

Further explanations

Quantification of 59122 maize and CV127 soybean are not accredited methods
The CV127 method is accredited only as a qualitative.
Accreditation for CV127 event well be done in the following months
The methods for detected event in sample T1 are not under accreditation in our laboratory.
The method is not in routine use and therefore not fully validated via certified reference material.
We are not accredited for quantification of 59122 and CV127
Not all events are covered
Method for CV127 has not been accredited.
Work was done according to our accredited method protocol
The method for CV127 is not accredited because it cannot meet our demands for verification
On routine, we only perform qualitative analysis of CV127 soybean, but not quantitative.
CV127 is not yet accredited. Some Screening PCRs are not yet accredited.
The quantitative method for CV-127 will be evaluated by the accreditation body at the next annual audit in February and will then be included in our scope of accreditation
Digital PCR as a new method to identify and quantify GMO is not yet accredited under ISO/IEC 17025:2017 in our laboratory. The accreditation is in progress and will be completed in 2023.

What was the approximate sample intake used for DNA extraction (in mg powder)?

	500 mg	400 mg	300 mg	200 mg	150 mg	100 mg	<100 mg
T1	16	0	5	40	2	2	0
T2	16	0	2	40	0	4	1

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	2
CTAB method with 2% CTAB in lysis buffer	18	19
CTAB + Maxwell 16 Food, Feed, Seed	5	6
NucleoSpin Food	18	17
NucleoSpin Plant	2	2
GeneSpin	5	5
Promega Wizard	2	2
Qiagen DNeasy Plant	0	0
Qiagen DNeasy Mericon Food	3	2
Biotecon Foodproof	2	2
SDS	2	1
Fast ID Genomic DNA	0	0
Generon Ion Force	1	1
Eurofins DNAExtractor cleaning column	2	2
Promega Wizard DNA clean-up resin	2	0
Qiagen QIAQuick	2	2
Qiagen Genomic-Tip 20/G	1	1
NucleoSpin gDNA clean-up	0	0

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

CTAB lysis with magnetic bead clean up (NucleoMag Plant)
QIAEX purification
Work was done according to our accredited method protocol

For maize we apply supernatant twice to get enough DNA-yield
Modified Qiagen Blood & Tissue kit
Increased amount of sample intake and volume of lysis buffer
Speedtools Food DNA Extraction Kit (Biotools)
We used the Macherey-Nagel NucleoMag Food with a KingFisher Flex extraction machine
Addition of amylase 40 µl (10 mg/ml) and proteinase K 40 µl (20 mg/ml)
CTAB precipitation method
Maxwell RSC PureFood GMO KIT; AS 1600
Extraction: Macherey-Nagel Nucleomag DNA Food kit
T1: Addition of alpha-Amylase
T1: SureFood Prep Advanced Kit + Purification T2: A CTAB lysis was performed prior to the DNA extraction with MN Food kit

Did you verify absence of PCR inhibition in the extracted DNA?

Answer	T1	T2
No	3	7
We performed a PCR inhibition test on a reference gene target prior to the analysis	28	25
We performed a PCR inhibition test on a GM gene target prior to the analysis	2	2
We analysed two or more dilutions of the DNA and compared the results	39	37
An internal 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

Spectrophotometric measurement of concentration and OD ratios: 3 answers
We used the Zymo OneStep PCR inhibitor Removal Kit for both DNA extracts.
Inhibition tests were performed on both samples prior to DNA quantification. T1 did not show inhibition. In T2, test was first done with ~120ng DNA (2 µl) and then with ~60 ng DNA (1 µl), and on both cases inhibition was present for T2. We did not want to reduce the DNA amount further, as the quantification analysis would suffer from less DNA (our accredited protocol recommends using 100 ng per qPCR repeat). If our quantification results are poor, we will try repeating the DNA extraction with a different method & purify DNA. However, as an alternative DNA extraction method (such as the CTAB method) is not accredited for our GMO analysis, we did not do so at this point.
Inspection of the amplification curves
DNA quality check: ratio of absorbence and in the course of the PCR - inhibition controls and at least two different DNA concentrations (if possible with 40 µg/µL and diluted 1:4). No inhibition could be detected for either samples.
We check that the ratios OD260/280 and OD 260/230 are acceptable.
The extracted DNA was diluted to 20ng/uL and a further 1:4 dilution was prepared. Both dilutions were amplified using a suitable reference gene qPCR assay (T1: hmg, T2: lec). The delta Cq-value was assessed for PCR inhibition (expected delta Cq +/-0.5). For both samples no inhibition was observed.
We measured DNA with a nanodrop and the relation A260/280 and A230/260 were both above 1.7. To verify the absence we made a dilution 1:10 of the sample and we performed a maize taxon gene analysis. We obtained a difference of 3.3 Ct´s (28,26 Vs 31.57) that is what we expect when there is no inhibition.
First we check general extraction success and inhibition by running a taxon specific PCR in 2 dilutions, followed by screening and event-specific PCR-methods, each with 2 dilutions (normally undiluted and 1:10)
We performed an inhibition test on the endogenous gene test of each DNA extract. This test is based on the comparison of the quantities between the pure DNA extracts (in the case of this EILA, otherwise we want 60000 copies) and 1/4 DNA extracts.
Approach described in the guide Verification of Analytical Methods ... Annex 2: Evaluation of DNA-extraction method (Inhibition test).
We used: negative and positive extraction and PCR controls, inhibition absence was tested with similar items (multigrain breads) in methods validation
3 consecutive 1/4 dilutions were applied and the delta Ct was assessed: inhibition was observed in undiluted T2, in T1 no inhibition was observed.
Absorbance 260/280 =1.8 in both items; inhibition test was performed using two PCR replicates using four points of a four-fold serial dilutions (1:4; 1;16;1:64; 1:256) of each DNA extraction replicate. All the acceptance criteria are met.

Do you consider the DNA extracted from T1 and T2 as suitable for quantitative PCR analyses?

	T1	Ratio	T2	Ratio
Yes	57	89.06%	57	89.06%
No	0	0%	0	0%
Not sure	1	1.56%	1	1.56%
Not applicable	3	4.69%	5	7.81%
No Answer	3	4.69%	1	1.56%

If screening methods were used, please indicate the results (presence or absence).

Screening target	T1: present	T1: absent	T2: present	T2: absent
P35S	54	1	1	21
tNOS	4	49	0	21
PAT	39	2	1	16
BAR	0	31	0	12
CP4-EPSPS	1	5	0	3
Ctp-CP4-EPSPS	0	3	0	2
Ctp2-CP4-EPSPS	6	21	0	12
Cry1Ab/Ac	4	13	0	6

Cry1Ab	0	1	0	0
pFMV	2	17	0	12
pNOS	0	3	0	0
t35S	3	0	0	0
nptII	0	8	0	5
p35S-pat	10	1	1	3
p35S-nptII	1	0	0	0
pCsVMV-pat	0	2	0	0
tE9	2	8	0	7
Agroborder I and II	3	1	0	0
CaMV	0	2	0	1
Other	2	3	0	0

Further details on other screening targets

T1: Event-specific real-time PCR DAS40278/VCO-01981-5/MON87419: < LOD (0.1 m/m) (events without p35S and/or tNOS); amplification signals for tNOS < LOD (0.1 %) (2x)
T2 sample: hmg, PLD, MON87701, MON87708, MON87769, 305423: absent
Very low Cq value for CTP2-CP4 EPSPS
The instructions for the T2 sample were to verify the presence of soybean CV127 and to quantify the CV127 content. We did not use screening methods for this reason.
PSP for Maize
To identify the GM event in T1, a screening was done using GM maize event-specific tetraplex qPCR assays.
Tnos and epsps are positive near detection limit (cts: 37-38)
T1 was further tested for 59122, TC1507, 88017, 87411, T25, 4114, 40278
Screening methods for T1 only were used with Real-Time-PCR. Positive GMO events were further quantified using digital PCR (true for T1 and T2).
P35S and pat were detected at levels close to LOD
We applied the prespotted plate for maize, and also performed event-specific detection methods for events that were not covered by the prespotted plate (4114, MON87411, MON87403, MZHG0JG, MZIR098)
T1: Agroborder I present, Agroborder II absent
We consider the use of the terms "Agroborder I" and "Agroborder II" as not correct, because these are trade names of kits and not screening elements. It will be more appropriate to list the genetic elements that are detected by these kits, and not the kits themselves. In this way the use of these kits will not be promoted by EURL.
DAS 40278-9 was absent
Actin, absent
T1: LY038, DAS-40278, VCO-01981, MON87419, DP-98140, MON810, MON87403, MON 87429, T25, DP-4114, TC1507, MON87411 all absent T2: CV127 only analysed
T1: pea DNA present

If your laboratory did not perform tests for all GM events (but only screening and/or some event-specific tests), which results would you report to your customer (Competent Authority or other)? Do you report the outcome of the screening tests applied (as indicated above) and/or indicate the GM events that were (or were not) analysed? Additionally, do you send the samples to another laboratory for further analysis?

T1: not applicable, tests for all GM events were/are performed in our lab
We would report all results from tests that the laboratory performed. Yes, we send the samples to another laboratory for further analysis.
The results of the screening are usually not communicated. The event is mentioned in the results report. If no event-specific procedures are established, the sample is forwarded to an accredited laboratory, e.g. as part of the country cooperation.
As a laboratory, we have about 70-80% reagents for identification. Whatever we find that is not accredited, we list the elements/strains found in the test report. We do not send samples to another laboratory.
We report the outcome of the screening tests applied (as indicated above) and indicate the GM events that were analysed. We do not send the samples to another laboratory for further analysis. (11 answers)
Our laboratory do not perform quantitative tests (when needed); in case of need, we foreward samples to Italian NRL. Final Report to Competent Authority is always completely filled with results of all tests performed by each involved laboratory
The laboratory performs the analysis for the official control - screening identification and quantification. We use PSP. We report screening results only on genetic elements and events that we have analyzed. In case of detection of GMOs, we carry out quantification. We do not send samples to another laboratory for additional analysis
The outcome of all test results available/requested are reported to customers. A second examination is only carried out in case of ambiguous results.
We performed screening + test of GM not covered by screening. If screening is positive, specific event is further search for. These are further quantified. Customers are informed about all assays performed (screening + specific tests).
T1: We did perform tests for all GM events (except MZIR098, which is currently under implementation). We would report that we found 59122 (0,94 +/- 0,8%)
We report the outcome of the screening test applied and of the GM events that were analysed. If it is necessary we send the samples to another laboratory for further analysis.
If the sample is considered compliant to EU regulation, we do not report any results to the customer (we just get "official" samples). If a sample is not compliant, the veterinary/food office gets a report, where every performed method is listed with its result.
We report the results from the screening as well as from event-specific tests analysed. We do not indicate events that were not analysed.
We perform analysis of set of screening markers (maize-sp. + transgenic markers in this case) and the GM events, coming as output

of the DSS as potentially present in the sample. The report contains only the tests that are performed and the respective result. Further, we send the results to our Control Authorities via a tool organised similar to the table here above: the performed tests and the respective results are filled in, the ones not tested are left empty.
We report the results obtained in the analysis of GM events and also of the screening PCRs. As technical information we report GM events that cannot be present in the sample as a result of the screening. Normally we do not send the sample to another laboratory.
We tested all known maize events for which a detection method is available. In the hypothetical case that is described here, we would report the outcome of the screening tests applied (as indicated above) and indicate the GM events that were analysed. We do not send the samples to another laboratory for further analysis.
We report outcome of the screening test applied and the GM events that were analysed (40-3-2, MON810 and Bt11)
ADH maize gene detected; lectine soybeen gene not detected; P35S promoter detected; Tnos terminator not detected; DAS 40278-9 element not detected; Bt11 maize event not detected; MON810 maize event not detected. Sample will be sent to the National Reference Laboratory.
Yes, I am reporting all screening test results to my costumer.
In case of positive screening markers, we report this and ask permission to the client for further event identification, and if positive events detected, quantification. Only in rare cases, for some clients, no further analysis is required but screening results only are sufficient. We do not send samples to another lab for further analyses; sometimes other labs send samples to our lab for event identifications/quantifications.
The laboratory reports only the test performed for the sample (screening and event-specific). If further investigation is required such as NGS analysis that can not be performed by our lab we send the sample to another laboratory.
The laboratory reports only the test performed for the sample (screening and event-specific). If further investigation is required such as NGS analysis that cannot be performed by our lab we send the sample to another laboratory.
My laboratory performs all GM events
We report the outcome of the screening tests applied and if necessary, identicate and quantificate of GM accredited events
We would report the results of all screening tests applied and report our conclusion about the events that cannot be present. We would not send the samples to other labs for further tests.
First screening; based on the screening result quantification of potential GMO events; not sent to another lab

Which master mix was used for T1 and T2 analysis?

Master Mix	**T1**	**T2**
2x TaqMan Universal PCR Master Mix (Applied Biosystems-Thermofisher)	17	16
ddPCR Supermix for Probes no dUTP (Bio-Rad)	3	4
Diagenode D600 for quantification.		2
PrimaQUANT qPCR PROBE MasterMix		1
Takyon Eurogentec No ROX	1	1
2x GoTaq Probe qPCR MasterMix (Promega)	1	1
PerfeCTaqPCR ToughMix or Fast Mix (Quantabio)	1	
Itaq Universal Probes Supermix (BIO-RAD)	1	
QIAcuity Probe PCR MX	1	1
Kapa Probe Fast qPCR Master Mix (2X)	1	1
IQSupermix (BioRad)	1	1
QP2X-03W0U - EUROGENTEC	1	2
LightCycler480 Probes master	1	1
LUNA Universal Probe qPCR MasterMix - New England Biolabs	1	1
Premix Ex Taq master mix for probe-based real-time PCR	1	
AmpliTaq Gold DNA Polymerase with Buffer II and MgCl2	1	

Provide the full code of the CRM used for quantification (for calibration or as QC material)

Target	**CRM code**	**Answers**
59122	ERM-BF424d	21
	ERM-BF424c	7
	ERM-BF424b	3
	ERM-BF424	3
	0306-H11	1
CV127	0911-D	4
	0911-C2	10
	0911-C	14
	0911-A	4
	pENGL-00-01/09-01	1

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

Test item	**Reference target**	**Answers**
T1 – 59122	Maize *hmg*	47
	Maize *Adh1* – 134/136 bp	4
	Maize *Adh1* – 70 bp	1
	Maize Invertase	1
	Other	0
T2 – CV127	Soybean *Le1* (74 bp) - QT-TAX-GM-002	44
	Soybean *Le1* (102 bp) - QT-TAX-GM-003	3
	Soybean *Le1* (81 bp) - QT-TAX-GM-001	4
	Soybean *Le1* (70 bp) - QT-TAX-GM-004	0

	Soybean *Le1* (118 bp) - QT-TAX-GM-007	1
	Other	3*

*QT-TAX-GM-005

Please enter the (average) slope of the calibration curves for GM and taxon targets in T1 and T2 (if applicable).

Test item	Target	Average	Minimum	Maximum	Number of data
T1	59122	-3.44	-4.13	-3.11	22
	Taxon	-3.43	-3.73	-3.20	21
T2	CV127	-3.41	-3.78	-3.20	20
	Taxon	-3.36	-3.95	-3.26	19

Provide details of any conversion factor used to convert your results for T1 and T2 from GM copy number ratio to GM mass fraction (e.g. when using dPCR).

dPCR?	Conversion factor
No	We used a factor of 2 between the mass/mass and copy/copy results [for T1]
No	T1 , CF= 0.34
No	For [qPCR] quantification plasmid calibrants were used (harbouring the reference gene and transgene target on a 1:1 ratio. Therefore, the conversion factors specific to CV127 and DAS-59122 indicated for ddPCR were used.
dPCR	For T2 1.01 conversion factor was used.
dPCR	T1 : 0,34 - T2 : 1
dPCR	T1 event DAS59122 conversion factor used: 0.34 and T2 event CV127 conversion factor used: 1.01
dPCR	CF(59122) = 0.34; CF(CV127) = 1.01
dPCR	CF for CV127 soybean=1.01, CF for 59122 maize=0.34
dPCR	T1: CF for CV127 := 1,01; T2: CF for DAS59122 := 0,34
dPCR	DAS59122 (CF = 0.34), CV127 (CF = 1.01)
dPCR	ddPCR T2: inhouse validated conversion factor 1.16
dPCR	CF for T1 (59122): 0.35; CF for T2 (BPS-CV127): 1.14
dPCR	We used in-house-validated conversion factors of 0.273 for T1 and 0.967 for T2

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?

For the answers, see Section 7.3.4 in this report.

Please justify the answers provided above (only the most informative answers are shown).

T1: EU authorised GM event 59122 maize, GM content including uncert. value above 0.9 m/m%; T2: EU authorised GM event CV127 soybean, GM content including uncert. value below 0.9 m/m %
"T1: The result for 59122 (EU authorized GM event) is 1.61 % (m/m) what is >0.9 m/m % (also after deducting the uncertainty), hence requiring labelling.
T2: The result for CV127 (EU authorized GM event) is 1.09 % (m/m) what is >0.9 m/m % but after deducting the uncertainty is less than 0,9 % and thus compliant to Reg. 1829/2003 - no labelling required. "
Because of different Legislation in our Country. [CNC]
Not relevant for Turkey [CNC]
The determined GM content (including measurement uncertainty) was in both cases above 0.9%.
Only qualitative tests were performed in the T1 sample. In sample T2 event CV127 is present at >0.9 m/m %, hence requiring labelling.
For T1, the threshold value of 0.9% is exceeded for DAS59122 even with consideration of the measurement uncertainty. The sample must therefore be labeled as "genetically modified". For T2, this cannot be conclusively assessed with reference to CV127, since the internal laboratory procedure has not been fully validated.
T1: the threshold of 0.9% is save exceeded; T2: the threshold of 0.9% is not save exceeded
T1 : GM% - MU > 0.9% ; T2 : GM% - MU ≤ 0.9%
"The concentration of GMOs in T1 is still greater than 0.9% with measurement uncertainty, so it is necessary to label it. The GMO concentration in T2 is less than 0.9% with measurement uncertainty, so it is not necessary to label it."
"T1: During the detection and identification of GMO in sample T1 we detected presence of one authorised maize event DAS 59122. From our measurement result and after the subtraction uncertainty from our result, value of result is above 0.9% - it means, that the sample is not compliant with the EU GMO legislation and labelling is required. T2: During the detection and identification of GMO in sample T2 we confirmed presence of authorised soybean event CV 127. From our measurement result and after the subtraction uncertainty from our result, value of result is below 0.9% - it means, that the sample is compliant with the EU GMO legislation and labelling is not required.
"Fortuitous presence of GM event CV127 authorised in EU (Food and Feed direct use or processing) and GM mass fraction measured is >0.9 %"
GM maize and soybean present above 0.9% after subtraction of measurement uncertainty in both samples, therefore it has to be labelled
T1 : DAS-59122 is authorized ; T2 : CV127 is authorized
"T1 contains 59122 2.07+/-0.21 m/m %, and that is >0.9 m/m %. T2 contains CV127 0.82+/-0.05 m/m %, and that is </= 0.9 m/m %."
"T1: The content of DAS59122 is 2.02%. The content is > 0.9 m/m %. The sample must be labelling. T2: The content of CV127 is 2.89%. The content is > 0.9 m/m %. The sample must be labelling"
T1 = 0.23 (U) below 0,9% ; T2 = 2,23 above 0.9 %
"T1: DAS59122 is allowed to be used in EU for food and feed, but not for cultivation. Thus the labelling limit of 0,9 m/m% applies --> As the product was not labelled as GMO, our result would render the sample not compliant to Regulation 1829/2003. If we take into account of our expanded MU (0.43), the quantification result does not fall below labelling limit (1,09 m/m%), and therefore we consider this sample to be non-compliant.

T2: CV127 is allowed to be used in EU for food and feed (phasing out), but not for cultivation. Thus the labelling limit of 0,9 m/m% applies --> As the product was not labelled as GMO, our analysis results (tested 2x) would render the sample not compliant to Regulation 1829/2003. However, if we take into account our expanded MU (0.43), the quantification result could also fall below labelling limit (with 0,78 m/m%). In addition, as the sample showed inhibition which we could not get rid off, there is a possibility that the sample would have a GM soy value higher than 1.37 m/m% - which would in turn affect the value that includes expanded MU, even over the labelling limit. (Please note, even when reduced the DNA template amount, our quantification results did not change, so we are not sure if the quantification result would increase considerably, but will have to see what the result of the PR round is). Thus our answer for T2 is: ""Cannot be concluded"". (I would seriously run more analysis for this sample if we just had the time)."
Both has > 0,9 % m/m, also after calculating the expanded uncertainty.
"Both detected GM-events have a valid approval. T1: The result for Maize 59122 is 1.99% +/- 0.60%. Subtracting the measurement uncertainty, the value is above the labelling threshold of 0.9%. The sample therefore does not comply with the legal requirements, it would have to be labelled as GMO. T2: The result for soybean CV127 is 1.46% +/- 0.44%. Subtracting the measurement uncertainty, the value is above the labelling threshold of 0.9%. The sample therefore does not comply with the legal requirements, it would have to be labelled as GMO."
Authorized GM corn DAS-59122-7 was detected in Test Item 1 and soybean CV 127 was detected in Test Item 2. The amount is higher than labelling threshold thus its presence must be labelled.
The samples are compliant when taking into account the measurement uncertainty
"T1: DAS59122 is authorised in EU and GM mass fraction mesured is higher than 0.9%.
T2: CV127 quantification analysis has not been performed"
T1 contains 59122 maize at 1.95 +/- 0.68 m/m %. For T2 we detected CV127 at a level of 1.12 +/- 0.39 m/m%. If the lowest value is taken into account (1.12 - 0.39) the sample is compliant to Regulation 1829/2003.
"T1: the amount of maize GM-event is >0.9 m/m %, therefore labelling is required (the identified event is authorized for use as food).
T2: the amount of soybean GM-event is >0.9 m/m %, therefore labelling is required (the identified event is authorized for use as feed)."
T1 and T2 have presence of GM events. Both events are authorised events, so we must look at regulation 1829/2003. In both samples, the amount of gm event present (quantification value - uncertainty) is below 0.9 m/m %, so labelling is not required.
If the measurement uncertainty is taken into account, both gmo events are present below 0.9 m/m % (59122: 0.94-0.15<0.9 / CV127: 0.99-0.26<0.9
Value for CV-127 in T2 is considered below 0,9% if taking into consideration the MU
"For T1: The quantification result is > 0,9 % even if applying the MU. For T2: The event CV 127 is approved for feed with restrictions. The restriction for the use as forage is not applicable for this sample, since it is soybean flour."
"T1: DAS59122 is approved for food and feed. The DAS59122 content is > 0.9 % in T1 and requires labelling. Hence, T1 is not compliant to Regulation 1829/2003. T2: CV127 is approved for food and feed. The CV127 content is < 0.9 % (taking Measurement Uncertainty into acount) in T2 and does not require labelling. Hence, T2 is compliant to Regulation 1829/2003."
"T1: The found value is (under consideration of the measurement uncertainty) above the threshold value of 0.9 % m/m . The event is approved for food, so labelling is required. T2: Under consideration of the measurement uncertainty (+/-0,22 % m/m),the found value (1,07 %m/m) can not be considered as being significantly above the labelling threshold (0,9 % m/m). So no labelling is required, if the presence is adventitious or technically unavoidable."
Our laboratory does not give compliance on the samples. The conformity is reserved to the competent office of the Ministry in charge of Agriculture.
"Result in T2 is above 0,9% but calculated with m.u. is equal 0,9%"
Results including (i.e. by subtracting) measurement uncertainty above 0.9% each
T1: the event is authorised and was detected below the LOQ of the method used (LOQ=0.1%). T2: the event is authorised and %GM - MU is below 0.9% (1.35-0.62=0.73)
"For T1: 59122 GM maize is 1.83 (minus U=0.57) = 1.26% of total maize, >0.9%. For T2: If routine sample, compliant cannot be concluded as only analyzed for CV 127 soya. Based in our measurement results CV127 GM soja is <= 0.9% (0.86)."
T1 sample contains >0.9 m/m % GM material, T2 sample contains >0.1 m/m % GM material
"T1: 1.55-0.67=0.88% DAS59122 maize. This is smaller than 0.9% and therefore compliant since DAS59122 maize is authorised. T2: 1.17-.037=0.80% CV127 soybean. This is smaller than 0.9% and therefore compliant since CV127 soybean is authorised."
"Maize 59122 is approved to be used for food purposes. The result minus uncertainty is below 0.9%. No labeling is required. Soy CV127 is approved to be used for food and feed purposes. The result minus uncertainty is below 0.9%. No labeling is required."
"T1: (1.78±0.52) % m/m >0.9% m/m. T2: (1.61±0.35) % m/m >0.9% m/m"
"T2: taking into account the measurement uncertainty, the threshold value of 0.9% is just undershot. If the addition is accidental and technically unavoidable, the GMO entry does not have to be declared.
T1: Clear exceeding of the threshold. According to the VO, the goods must be declared as GMO"
Both above threshold of 0.9%
1,03 % +/- 0,17 - CV127 may be present at level below 0,9%.
Both events are EU authorized and contain the GM event above 0.9% m/m% (considering expanded uncertainty) so labelling is required.
"The 59122 is an authorised GM event in the EU, hence the labelling threshold to be applied is 0.9 m/m%. Our result was 1.85 m/m%. 1.85 m/m%-MU>0.9%, so T1 is not compliant, should have been labelled. The CV127 is an authorised GM event in the EU, hence the labelling threshold to be applied is 0.9 m/m%. Our result was 1.34 m/m%. 1.34 m/m%-MU>0.9%, so T2 is not compliant, should have been labelled."
T1 is consider not compliant because the GM event detected/ quantified was >0.9m/m%. T2 is consider compliant because the GM event detected/ quantified minus the uncertainty is <0.9 m/m %
T2 is consider compliant because the GM event detected/ quantified minus the uncertainty is <0.9 m/m %
both events are authorized, but DAS59122 event in T1 is >0.9 m/m % considering the lower limit of the measurement uncertainty and CV127 event in T2 is <0.9 m/m % considering the lower limit of the measurement uncertainty
With 95% confidence, the GM content is above the labelling threshold of 0.9% m/m.
CV127 and DP-59122 do not fall under the regulation 619/2011. The Content of CV127 and DP-594122 are > 0,9 m/m %.
"T1: We have detected maize DAS59122 which is authorized for food according to Regulation 1829/2003. The reported maize DAS59122 content is 1,57+/-0,26 [m/m%] which is above labelling threshold therefore sample should be labelled as GM food.

T2: We have detected Soybean BPS-CV127-9 which is authorized for feed according to Regulation 1829/2003. The reported Soybean BPS-CV127-9 content is 0,99+/-0,13 [m/m%] which is below labeling treshold assuming that the Soybean BPS-CV127-9 presence adventitious or technically unavoidable."
"T1: result including measurement uncertainty is above 0,9 m/m%; T2: result including measurement uncertainty is below 0,9 m/m%"
Taking account of measurement uncertainty the result of sample T1 is above 0,9%m/m and the result of sample T2 is below 0,9%m/m.

Additional comments and suggestions

The analysis of T2 was performed by Chemical and Veterinary Analytical Institute Rhein-Ruhr-Wupper (cooperating institute).
Only qualitative RT PCR was used
It would be necessary to have a pt with unauthorized GMO: flax FP967.
The quality of the AOCS CRM in not good. Apparently does not appear homogeneous.
Nice round, thanks. Am quite eager to hear about the T2 inhibition.
Extra grinding step of AOCS-Reference material with Retsch MM 400 mill was necessary because the material was not fine and homogeneous.
Please don't send such complicated samples before Christmas holidays!
The answers referred to the technique of Digital PCR.

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