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EURL European Union Reference Laboratory for GM Food & Feed

Determination of GM Maize in Bakery Mix (T1) and GM Oilseed Rape in Rapeseed Meal (T2)

GMFF-22/01 Proficiency Test Report

BELAC 268-PT

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

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Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised the proficiency test (PT) "GMFF-22/01" for the determination of GMOs in food or 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. T1 consisted of a blank (i.e. non-GM) material produced from a commercial mixed flour for the preparation of multigrain bread. T2 was composed of ground rapeseed meal spiked with GM oilseed rape events MS8 (Unique Identifier ACS-BNØØ5-8) and RF3 (ACS-BNØØ3-6). These two GM events are usually part of a stacked GMO (MS8xRF3). The EURL GMFF evaluated the homogeneity and stability of the test items and derived the assigned values from in-house measurements.

Sixty-three laboratories participated to the PT round, consisting of 53 NRLs from 24 EU Member States, 5 EU OCLs and 5 official testing laboratories 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, as expected for a blank material, none of the laboratories reported the presence of a GM event. For T2, all laboratories except two NRLs and one OCL (95 %) detected both MS8 and RF3.

The quantitative results reported for T2 were evaluated using z (for MS8) or z' (for RF3) 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, 9 did not report any quantitative results, while 7 reported truncated values (less or greater than). The vast majority (over 80 %) of the other laboratories proved their satisfactory performance (expressed as z or z' score) for the analysis of MS8 and RF3 in rapeseed meal. All participants (except one) reported their measurement expanded uncertainty and coverage factor associated with their respective measurement values. More than 69 % properly assessed the compliance of the two test samples investigated.

The evaluation of this PT round confirms that most NRLs and OCLs are able to monitor mass fractions of GMOs in rapeseed meal in the frame of Regulation (EU) 2017/625.

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

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 **detection of GMOs in a bakery product and for the determination of the mass fractions of MS8 and RF3 oilseed rape in rapeseed meal,** 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 samples were prepared and dispatched to participants for analysis. A bakery mix blank material (food test item T1) was selected to resemble the majority of the food products analysed by control laboratories in the EU, in which generally no GMOs are detected. The second sample (feed test item T2) consisted of commercial rapeseed meal spiked with seed powder of two oilseed rape events, MS8 and RF3. The stacked GMO MS8xRF3, commercialised under the trade name SeedLink® oilseed rape, provides a pollination control system, linked to a herbicide tolerance trait.

This report presents the outcome of the PT.

2 Scope

The present PT round 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-22/01**".

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-22/01 exercise was announced, by invitation letters to NRLs and some accepted non-EU OCLs, on April 25, 2022 (Annex 1). The registration deadline was set to May 6, 2022. Samples were

sent to participants on May 24, 2022. The deadline for reporting of results was set to July 8, 2022. This deadline was extended to July 12, 2022.

3.4 Distribution

Each participant received:

- One bottle of test item T1 (bakery mix), containing approx. 5 g of dry powder;
- One bottle of test item T2 (rapeseed meal), 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 individualised "Instructions letter" (Annex 3), sent by email on the day preceding the dispatch.

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 – Bakery 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 – Rapeseed meal (for feed):

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

Participants were requested to apply their routine approaches for GMO testing. The quantitative results had to be expressed in mass/mass % and applying the rounding that they would normally apply when reporting to their customers. Attention was requested to the correct estimation and reporting of the measurement uncertainty ($U(x_i)$) and coverage factor (k) used. Since the homogeneity study was performed with 200 mg sample intake for T1 and T2, the recommended minimum sample intake was set to these amounts.

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 any GM event 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 cryogenically milled using a Palla VM-KT vibrating mill, Humboldt Wedag, (Colone, DE). Thereafter the powder was sieved over a 710 µm stainless steel sieve (Russel Finex, London, UK). An additional drying step was applied as the resulting powder initially had a water content of 13.2 % (m/m). Following a vacuum drying step in a freeze dryer (Epsilon 2-10D from Martin Christ, Osterode DE), the resulting powder was homogenised in a 3-dimensional Dynamix CM200 for 1 h.

The resulting powder 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 100 vials were produced. The final water content and particle size is reported in Table 1.

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

Characteristic	Bakery mix
Type of base material	Crude powder
Origin	Local grocery AVEVE (BE)
Grinding equipment	Cryo-grinding vibrating mill
Mixing equipment	Dynamix CM200
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	4.5 ± 0.1
Particle diameter in µm, mean ± *U*[1] (*k*=2, *n*=3)	61.1 ± 10.8
Mass used to prepare T1 (g)	2400

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

Test item T2 consisted of ground rapeseed meal, purchased from a local rapeseed oil producer in Belgium. Rapeseed meal (also called rapeseed cake) is the high-protein by-product of the extraction of oil from rapeseed, used as feed, e.g. for livestock and poultry. The bulk meal received from the producer was ground by cryogrinding (Palla VM-KT vibrating mill, Humboldt Wedag, Colone, DE). The presence of traces of maize and soybean was previously reported, as well as the presence of MON87701, MON89788 and GTS 40-3-2 soybean at low levels [9].

The meal was spiked with seed powders of GM oilseed rape events MS8 and RF3, kindly received from BASF for the preparation of the PT test materials. According to the company, the GM seed powders had the following characteristics:

- MS8 seed powder was partly hemizygous: seeds were harvested from female plants harboring one MS8 copy and pollinated by male plants with no MS8 copy; this means that 50 % of the harvested seeds contained one MS8 copy, the other 50 % was non-GM;
- RF3 seed powder was 100 % homozygous.

The official CRMs for these events (AOCS 0306-F8 and 0306-G7), consisting of leaf tissue DNA, are produced from hemizygous (MS8) or homozygous (RF3) plants. The zygosity of the CRMs and seed powders was confirmed by in-house ddPCR measurements (MS8, RF3 and *FatA(A)*). Applying the corresponding conversion factors, determined using *FatA(A)* as taxon-specific reference target (0.456 for MS8 and 0.907 for RF3), the seed powders used for spiking were (as expected) around 50 % (m/m) and 100 % (m/m) GM for MS8 and RF3 in comparison with their CRMs, respectively. The ddPCR measurements also confirmed that the number of *CruA* copies in these materials was two times higher than the *FatA(A)* copy number, which is consistent with

the presence of two *CruA* genes in *Brassica napus* (located on the A and C genome) compared to one *FatA(A)* copy (on the A genome) [10] .

The rapeseed meal powder was mixed with MS8 and RF3 seed powders, the mixture cryogrinded, mixed again afterwards, and filled in 5 g portions into 20 ml vials, closed under argon. The final water content and average particle size are reported in Table 2.

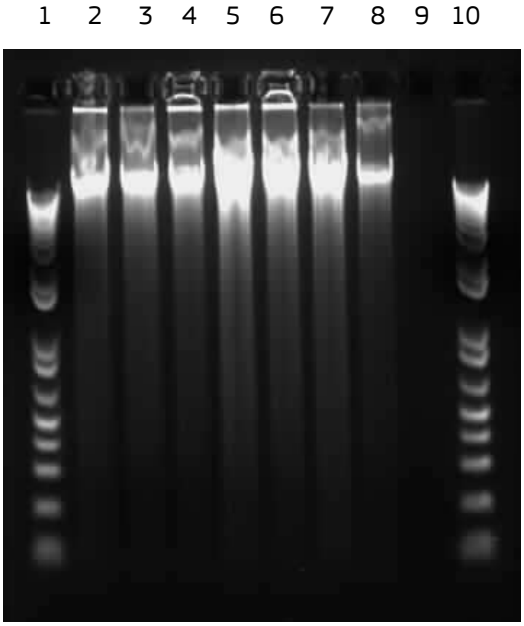
The amount and the quality of the DNA extracted from the T2 material using a CTAB method with Genomic-tip20 purification were verified by UV spectrometry, fluorometry and gel electrophoresis (Figure 1). A selection of DNA extracts were tested for inhibition with the *FatA(A)* target using serial dilutions and passed the evaluation criteria (slope and ΔCq).

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

Characteristic	**Rapeseed meal**	**MS8 seed powder**	**RF3 seed powder**
Type of base material	Crude powder	Ground seed powder, 50 % hemizygous GM	Ground seed powder, 100 % homozygous GM
Origin	Local oil-producing company (BE)	Delivered by BASF	Delivered by BASF
Grinding equipment	Cryo-grinding vibrating mill		
Mixing equipment	DynaMIX CM-200		
Mass used to prepare T2 (g)	680.40	9.80	9.80
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	3.3 ± 0.5		
Particle diameter in µm, mean ± *U*[1] (*k*=2, *n*=3)	90.3 ± 2.7		

[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 1. Agarose gel electrophoresis of genomic DNA extracted from the T2 material (lanes 2-8, lane 9 is 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 *FatA(A)* as taxon-specific reference target), 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. This was done for T2 only, as T1 was a blank material containing none of the measurands (i.e. maize GM events) to be investigated. Seven bottles were randomly selected and the extracted DNA (CTAB/tip20) was analysed by qPCR in 5 replicates each (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 both using the software SoftCRM v2.0.21 [11] and the excel calculation template in our quality system. The T2 material proved to be homogeneous for the GM event (Annex 4.1).

The stability during dispatch conditions was also only assessed for T2. It was performed using an isochronous short-term stability scheme [12] involving two test samples with three replicates each (N=2, n=3) and conducted over one week (3 and 7 days incubation) at +40 °C. The measurements by qPCR (MS8 and RF3) were performed under repeatability conditions. The results revealed no significant influence of storage at +40 °C on the stability of the test item (compared to storage at -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 as well only tested for T2 (T1 being a blank material) using qPCR, analysing the GM content (MS8 and RF3) in bottles (N=2, n=3) stored at the normal storage temperature (+4 °C). 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 T2 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 analytes [3].

5 Assigned values and corresponding uncertainties

5.1 Assigned values

The assigned values (x_{pt}) for the mass fraction of the MS8 and RF3 events in T2 were derived from measurement results obtained in two independent EURL GMFF laboratories by qPCR and ddPCR applied to DNA extracted by the CTAB or CTAB/tip20 methods (Table 3). The nominal fraction of MS8 and RF3 in T2 was 1.4 m/m %. The amount measured was lower than the gravimetrically prepared fraction due to the fact that the rapeseed meal (with oil extracted) contains more DNA per weight compared to the spiked seed powders. It was also expected that approximately two times less MS8 was measured compared to RF3 because only half of the MS8 seeds were GM seeds (hemizygous, but also the CRM is made from hemizygous plants), while the RF3 powder was pure GM (homozygous, as is the CRM).

The average RF3 content measured by laboratory 2 was considerably lower than the results of several datasets obtained in laboratory 1, but no technical reason was identified that would justify removing the former results and individual data overlapped between most datasets. This obviously increased the measurement uncertainty on the assigned value.

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}	$u(x_{pt})/\sigma_{pt}$
T2	MS8	qPCR (N=35) [1]	0.55 ± 0.06	**0.57**	0.03	0.01	**0.03**	**0.14**	0.21
		qPCR (N=15) [1]	0.55 ± 0.11						
		qPCR (N=15) [2]	0.53 ± 0.11						
		ddPCR (N=15) [1]	0.66 ± 0.14						
	RF3	qPCR (N=35) [1]	1.36 ± 0.18	**1.21**	0.14	0.04	**0.14**	**0.30**	0.47
		qPCR (N=15) [1]	1.45 ± 0.32						
		qPCR (N=15) [2]	0.83 ± 0.15						
		ddPCR (N=15) [1]	1.21 ± 0.15						

[1] Laboratory 1 used a CTAB/genomic-tip20 DNA extraction method
[2] Laboratory 2 used a CTAB DNA extraction method without column 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 mean values per dataset obtained by the expert laboratories and "*p*" refers to the number of datasets.

5.3 Metrological traceability of the assigned value

All values are traceable to the SI unit as a result of the use of CRMs with certified values traceable to the SI unit.

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 as follows: D=detected, ND=not detected, NT=test item or GM event not tested. This information had to be reported in the questionnaire accompanying the PT. 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 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.

According to ISO 13528:2015 [3], when $u(x_{pt}) > 0.3\ \sigma_{pt}$ (cf. RF3, Table 3) the uncertainty of the assigned value ($u(x_{pt})$) can be taken into account by expanding the denominator of the z score and calculating the z' score, as follows:

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

The interpretation of the z, z' and ζ performance scores is done according to ISO 13528:2015 [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* and *z'* scores** compare the participant's deviation from the assigned value with the standard deviation for proficiency test assessment (σ_{pt}) (or for z' the combined standard deviation for proficiency assessment (σ_{pt}) and the uncertainty of the assigned value) 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 [13].

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 pointed out that "$u_{max,rel}$" is a normative criterion when set by legislation, however, this is not specified in the GMO legislation.

It should be understood that the reported data from participants were not $\log_{10}$-transformed prior to the performance assessment [14].

7 Evaluation of reported results

7.1 Participants

Fifty three NRLs from 24 EU Member States (excluding Estonia, Malta and Ireland; the latter designated Wageningen Food Safety Research in The Netherlands as NRL for GMO analysis) and 10 OCLs registered to this PT round (Table 4). NRL/625 represented 56 % of all participants. All but a few laboratories reported qualitative results. In addition, 45 or 46 (out of 63) laboratories reported quantitative results for MS8 or RF3, respectively. Eleven laboratories, including five NRL/625, did not provide any quantitative data and seven laboratories, including three NRL/625, provided truncated values (see 7.3.2).

The majority of participants applied real-time PCR, while 4 (MS8) and 3 (RF3) laboratories reported digital PCR results. The experimental details are presented in Annexes 5 and 6.

Table 4. Overview of participants to GMFF-22/01 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	1	1		
Germany	18	1	15	2
Greece	1	1		
Hungary	2	1		1
Ireland	0	0		
Italy	3	1	2	
Latvia	1	1		
Lithuania	1	1		
Luxembourg	1	1		
Malta	0	0		
Netherlands	1	1		
Poland	4	4		
Portugal	1	1		
Romania	1	1		
Serbia	2			2
Slovakia	2	2		
Slovenia	1	1		
Spain	5	3		2
Sweden	1	1		
Switzerland	2			2
Turkey	1			1
Total	**63**	**35**	**18**	**10**

7.2 Qualitative results

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**, which was a blank material not containing any GM event, none of the 62 laboratories that had tested the sample detected any GM positive material. This confirms that GMO laboratories are correctly reporting negative results for materials devoid of GMOs (below the LOD). A number of laboratories admitted that not all GM events had been tested and were therefore unsure about the absence of any GMOs.

For **T2**, MS8 and RF3 are the only authorised oilseed rape GM events containing *tNOS* and *bar* elements and not any of the other common screening markers. All other oilseed rape events could therefore be excluded, except event 73496, which does not contain any screening marker. The evaluation of the screening results was complicated due to the traces of soybean GM events in the test item. A total of 95 % of the laboratories detected both MS8 and RF3. Two of the three laboratories that have not detected these events were NRL/625. A number of laboratories also reported the presence of one to three soybean GM events that were previously also detected in the rapeseed meal by the EURL GMFF [9]. The qualitative results are summarised in Table 5, while the individual laboratory results are presented in Annex 5.

The majority of laboratories demonstrated their capacity to identify the correct GM event in the test matrix.

Table 5. Qualitative identification of the GM events in T1 and T2

Test item and/or GM event tested?	Outcome	Any GM event in T1	MS8 in T2	RF3 in T2
Tested	Detected (D)	0	58	57
	Not detected (ND)	62	3	3
Not tested (NT)		1	2	3
Total		63	63	63

7.3 Quantitative results

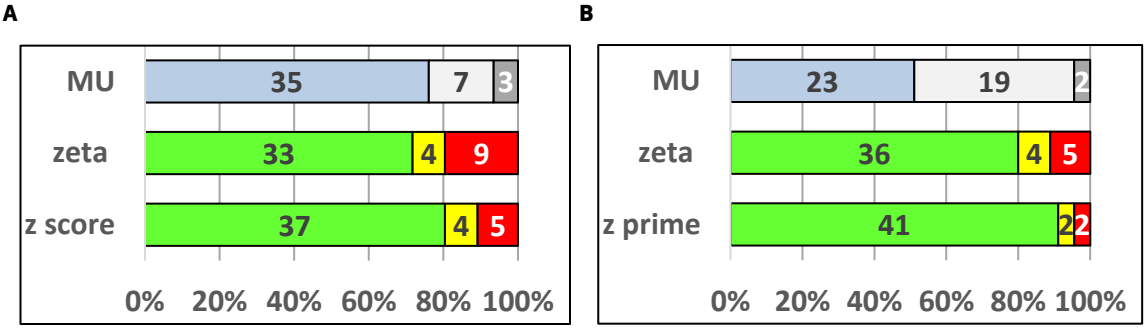
7.3.1 Performances

Laboratory performance for quantification of the GM events in test item **T2** was expressed in terms of z (or z') and ζ scores. Annex 5 presents the reported results as tables and graphs for each measurand. Satisfactory performance scores are 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 [15].

Figure 2 summarises the performance scores obtained. A total of 45 and 46 quantitative results were reported for MS8 and RF3 oilseed rape in T2, respectively, and have been scored. An overall satisfactory performance of over 80 % (MS8) and over 91 % (RF3) was obtained. Five and two unsatisfactory results were obtained for MS8 and RF3, respectively. The higher satisfactory performance for RF3 compared to MS8 may be related to the use of z' scores for RF3, which are less stringent due to the inclusion of the $u(x_{pt})$ in the denominator of the equation, which therefore becomes larger (and z' at the same time lower). When taking into account the reported measurement uncertainties, 9 (MS8) and 5 (RF3) results were determined as unsatisfactory (expressed as ζ score). The unsatisfactory $z(')$ scores were all overestimations of the GM content, up to 7.5 times by L13.

The 4 laboratories that had used dPCR (one of which only reported a value for RF3) all obtained satisfactory results, except one questionable result for MS8 by L42. L42 used *CruA* as reference target, and divided the CFs by two to account for the double *CruA* target compared to *FatA(A)* (on the basis of which the CF were calculated). However, in the questionnaire they switched the CF for MS8 and RF3, reporting the highest value for MS8. This may explain their questionable score for this event.

Figure 2. Overview of laboratory performance according to z and ζ scores, for the content of the oilseed rape events MS8 (A) and RF3 (B) in test item T2. 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

Seven truncated values were reported for both MS8 and RF3 in T2, most of which were "more than x" results (ranging from "0" to "0.045"). 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.

Only L11 reported "less than 0.1", meaning that the laboratory was not able to detect the GM events.

7.3.3 Measurement uncertainties

All laboratories having reported quantitative results, except L33 (OCL), provided expanded measurement uncertainties and coverage factors for both measurands (Annex 5). The missing uncertainty of L33 was shown as "not provided (np)" in the tables in Annex 5.

Most of the laboratories (76 %) reported a realistic measurement uncertainty (Case "a" in Figure 2) for MS8. For RF3, this percentage dropped to 51 %, which was expected as the larger $u(x_{pt})$ for RF3 (see Section 6) means that more of the reported $u(x_i)$ were smaller than this value (hence Case "b"). In this case, it does not necessarily imply that these laboratories have underestimated their measurement uncertainty.

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.

A total of 52 and 58 laboratories assessed the compliance of T1 and T2 samples, respectively. Most of them provided a justification for their choice among the 5 compliance options. The option selected and the justification provided were evaluated. 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.

As **T1** did not contain any GM events, the compliance statement should be CNL, i.e. the sample is compliant because no labelling is required. In routine control samples, laboratories may encounter blank samples. Moreover, the content of a specific species, and therefore the practical LOD, may be too low for accurate quantification of any GM events that may be present. The majority of laboratories (36 out of 37, excluding the 15 inconclusive answers) reported a correct compliance statement (Table 6). Four laboratories additionally answered C<LLP (and one laboratory only selected this answer), 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. Hence, a product can either be:

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

Several laboratories were unsure about the compliance of the sample (hence reported CNC) because no GM events had been detected, no quantification had been done or because the practical LOD was too high for accurate quantification (and not all potential events were tested).

Table 6. Reported compliance statements for T1 (blank bakery mix)

Compliance Statement	Laboratory Measurement	Number of Laboratories [a]	Comment
CNL - Compliant, because no labelling required	$X \pm U \leq 0.9$ m/m %	36	Correct, no GMOs detected
NCL - Not compliant, should have been labelled	$X \pm U > 0.9$ m/m %	0	
	$X \pm U \leq 0.9$ 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 %	5	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 %	0	
CNC - Cannot conclude / not quantified		15	
Total no. of participants that provided a statement		52	

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

The two GM events present in **T2** are authorised in the EU (even while their renewals are ongoing), 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 MS8 measured in T2 is below the threshold.
- The lower limit of the assigned (expanded) range for RF3 is 1.21 – 0.28 = 0.93 m/m %. Taking the number of significant figures in the legislation into account, the measured value needs to be rounded to 0.9 m/m %. Hence, labelling is not required for this material.
- On the basis of the measurement results obtained in the laboratory it is possible that $x - U > 0.9$ m/m %, in which case the sample should be considered not compliant to Regulation (EC) 1829/2003 because labelling is then required (CNL).
- Measurement results need to be reported per single event per species (cf. Report of the 30th ENGL meeting: https://gmo-crl.jrc.ec.europa.eu/ENGL/docs/ENGL-Plenary-30th.pdf).

Since two oilseed rape GM events are present in the sample, summing up of their contents may have been considered. The combined presence of both events (1.21 + 0.57 = 1.78 m/m %) would definitely exceed the threshold for labelling (taking into account the combined uncertainties, i.e. calculating the squareroot of the squared individual uncertainties, here $\sqrt{(0.28^2 + 0.06^2)} = 0.29$). However, because MS8 and RF3 are usually commercialised as a stacked product, it may be considered that summing up of the GM contents measured for these events may in this case not be justified for evaluation of the labelling requirement. It shows that whether the contents of the individual GM events are considered or the sum of their contents (per species or ingredient) leads to different conclusions about compliance.

Table 7 summarises the statements reported for T2, taking into account the reported analytical results (or lack of results). The majority of the laboratories (41 out of 42, excluding the 16 exclusively non-conclusive answers) correctly interpreted the compliance rules based on their obtained measurement results, with 34 concluding non-compliance if not labelled. Some laboratories (21 out of 41, i.e. 51 %) justified their answer by referring to the combined GM content obtained for MS8 and RF3, taking into account the combined uncertainties. Six laboratories (additionally) reported "(not) compliant under Regulation (EU) 619/2011", in some cases referring to the ongoing renewal of the authorisation; however, also during the renewal phase the GM event remains authorised. When disregarding the statements related to Regulation (EU) 619/2011, only the "NCL" statement of L44 is considered incorrect, as the justification referred to the summing up of the GM contents (0.43+0.75=1.18), without taking into account the combined uncertainties ($\sqrt{(0.18^2+0.47^2)}=0.50$, hence 1.18-0.50=0.68, which is ≤0.9).

Table 7. Reported compliance statements for T2 (rapeseed meal)

Compliance Statement	Laboratory Measurement	Number of Laboratories [a]	Comment
CNL - Compliant, because no labelling required	$x - U \leq 0.9$ m/m %	7 [b]	Correct (added up or not)
NCL - Not compliant, should have been labelled	$x - U > 0.9$ m/m %	12	*"At least one of the GM events is present above the labelling threshold"*
	$x - U \leq 0.9$ m/m %	0	
	$(x_i+y_i) - \sqrt{(U^2_{xi}+U^2_{yi})} > 0.9$ m/m % [c]	21	*"Sum of both events is above the labelling threshold"*
	$(x_i+y_i) - \sqrt{(U^2_{xi}+U^2_{yi})} \leq 0.9$ m/m % [c]	1	Combined uncertainties not correctly taken into account
C<LLP - Compliant, under Regulation 619/2011 but ≤0.1 m/m %, in feed	$x - U \leq 0.1$ m/m %	3	Wrong as this Regulation does not apply (not feed, no GM events listed as pending authorisation)
NC>LLP - Not compliant, under Regulation 619/2011 and >0.1 m/m %, in feed	$x - U > 0.1$ m/m %	3	
CNC - Cannot conclude / not quantified		18	
Total no. of participants that provided a statement		58	

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

[b] Four of these laboratories concluded CNL, despite the sum of the GM events (+ combined uncertainties) being above 0.9 m/m %

[c] x_i and y_i refer to the quantity measured for MS8 and RF3, respectively, which are added up, taking into account the combined uncertainties ($\sqrt{(U^2_{xi}+U^2_{yi})}$). The number mentioned here indicates the laboratories that have justified their compliance statement by clearly referring to the adding up of the results.

7.4 Questionnaire

The questionnaire was answered by all participants and gave valuable information on the laboratories, their way of working and their analytical approaches.

The majority of participants (70 % for T1 and 62 % 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 or no accreditation for the sample matrices.

Most laboratories (57) used screening methods for T1 and T2 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. Surprisingly, 12 laboratories identified p35S and tNOS in T1, which could be due to *Agrobacterium* contamination as some laboratories indicated.

GM event quantification was usually done by qPCR with standard curves, while 4 laboratories used the delta Cq approach for T2 and 4 laboratories used dPCR for T2. The most common master mix used in qPCR (34 laboratories) was the 2x TaqMan Universal PCR Master Mix (Applied Biosystems-Thermofisher), while 16 other master mixes were used by the remaining laboratories.

The CRMs from AOCS were used for calibration, although different batch numbers, some of which were purchased more than 5 years ago, were mentioned for both MS8 CRM (0306-F8 was the most recently released batch code at the time of the PT measurements) and RF3 CRM (0306-G7). *CruA* was the most often used endogenous reference gene target for T2 (37 laboratories), followed by *FatA(A)* (10), *PepC* (4) and *Ccf* (1). The individual slopes of the calibration curves for MS8 (average -3.40), RF3 (-3.45) and the different reference targets (-3.48) were in most cases within the acceptance limits of the Minimum Performance Requirements of the EURL GMFF (See Guidance documents under https://gmo-crl.jrc.ec.europa.eu/guidance-documents). The 4 laboratories that applied dPCR used a conversion factor (CF) to express their results in m/m % (taken from the CF list on the EURL GMFF website or in-house determined); three laboratories used *FatA(A)* as reference target (on the basis of which the CF were measured), while one laboratory used *CruA* and divided the suggested CF by two to account for the double *CruA* copy in the *B. napus* genome. Two other laboratories (L27 and L47, using *PepC* or *CruA* as reference targets) also reported a CF, however, while using qPCR; it seems that their results may have been overestimated as result of applying these CF.

Further information is available in Annex 6, which summarises all experimental details and comments provided by the participants.

8 Conclusions

The proficiency test GMFF-22/01 was organised to assess the analytical capabilities of EU NRLs and OCLs to test a blank food material (T1) and report the outcome and to determine the content of MS8 and RF3 oilseed rape in commercial rapeseed meal (T2), commonly used as feed material.

The vast majority of participants correctly reported the blank material as free from GMOs and identified both spiked GM events in the feed material T2, while 71 % of laboratories quantified these GM events. The overall performance of the participants for the determination of the content of both GM events in T2 was satisfactory (80 % for MS8, 91 % for RF3).

The compliance statements provided by most of the laboratories were considered in line with the results obtained 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 [16].

Acknowledgements

The laboratories listed hereafter are kindly 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
Executive Environment Agency	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
Landesuntersuchungsanstalt für das Gesundheids- und Veterinaerwesen Sachsen	GERMANY
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe	GERMANY
Landeslabor Schleswig-Holstein	GERMANY
Thüringer Landesamt für Landwirtschaft und Ländlichen Raum	GERMANY
LUFA Speyer	GERMANY
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GERMANY
Bavarian Health and Food Safety Authority (LGL)	GERMANY
Thüringer Landesamt für Verbraucherschutz	GERMANY
LAVES-LVI BS/H	GERMANY
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V (LALLF MV)	GERMANY
Federal Office of Consumer Protection and Food Safety	GERMANY
Landesamt für Verbraucherschutz Sachsen-Anhalt	GERMANY
LTZ Augustenberg	GERMANY
Landesuntersuchungsamt	GERMANY
Landeslabor Berlin-Brandenburg	GERMANY
German Federal Institute for Risk Assessment	GERMANY
Institute for Hygiene and Environment	GERMANY
CVUA Freiburg	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 Superiore di Sanita' (ISS)	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
National Veterinary Research Institute	POLAND
Plant Breeding and Acclimatization Institute NRI	POLAND
Regional Laboratory of Genetically Modified Food	POLAND
Instytut Zootechniki Panstwowy Instytut Badawczy Krajowe Laboratorium Pasz	POLAND
INIAV	PORTUGAL
Institute of Diagnosis and Animal Health	ROMANIA
SP Laboratorija a.d.	SERBIA
A Bio Tech Lab ltd	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. AESAN	SPAIN
Generalitat de Catalunya	SPAIN
SeLyC	SPAIN
Laboratorio Arbitral Agroalimentario - MAPA	SPAIN
Laboratorio Central de Veterinaria	SPAIN
Swedish Food Agency - Livsmedelsverket	SWEDEN
Federal Food Safety and Veterinary Office FSVO	SWITZERLAND
National Food Reference Laboratory	TURKEY

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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

Geel, 25 April 2022
JRC.F.5/UV/wb/mt/ARES(2022) 22- 026

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 proficiency test GMFF-22/01

Dear Colleague,

Hereby, I would like to invite you for participating to the proficiency test (PT) GMFF-22/01, organised by the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) in line with its mandate under Regulation (EU) 2017/625.

Participation to this PT is free of charge. Please remember that participation is mandatory for all NRLs designated under Regulation (EU) 2017/625 and recommended for NRLs nominated under Regulation (EU) No 120/2014. 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. These laboratories will have to register for this PT using the registration details provided in this letter.

This PT will include two ground test materials that will be dispatched at room temperature. They are processed by the JRC and "*derived from products that are not declared as containing GM material*". The testing laboratories are requested to check for the presence of GMOs and to assess the compliance of the samples with the applicable GMO legislation.

The following tasks are requested from the participants:

Test Item 1 – Multigrain bread dry 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 – Rapeseed meal (feed) (5 g dry weight):
- Verify the presence of GM oilseed rape in this sample;
- Quantify the GM event(s) identified and assess compliance of the sample.

Participants are requested to apply their routine approaches for GMO testing and to provide further details in a questionnaire via an online EU Survey.

The quantitative results have to be reported in mass/mass %. The EURL GMFF will calculate performance scores (z and ζ scores) for the reported results. Be aware of the existence of an appeal procedure in case you disagree with your scores.

Information on the identity of the participants in this PT will be kept confidential. However, the lab codes of the NRLs that have been designated in line with Regulation (EU) 2017/625 may be disclosed to DG SANTE for evaluation of their performance. Upon request from an NRL in a Member State, the lab codes of the official laboratories (or NRLs) within its network of control laboratories may also be disclosed to the NRL.

Please register electronically using the following link: https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=2761.

After registration, you are requested to return the signed registration form as scanned pdf to us by e-mail. Each laboratory can register only once for this PT.

The deadline for registration is set to **Friday 6 May 2022**.

The test items will be shipped on 24 May 2022. You are requested to inform us promptly if you have not received the samples by Tue 31 May 2022.

The deadline for submission of the results is set at Friday 8 July 2022.

Please contact the functional mailbox JRC-EURL-GMFF-CT@ec.europa.eu for all issues related to this PT.

Yours sincerely,

e-signed

Dr. Ursula Vincent, Head of Unit

Cc: Wim Broothaerts, PT coordinator

Contact:

European Reference Laboratory for GM Food and Feed
Dr Wim Broothaerts, Project leader GMO Control
Joint Research Centre of the European Commission
Retieseweg 111, B-2440 Geel, Belgium
Tel: +32 14 57 16 12; JRC-EURL-GMFF-CT@ec.europa.eu

Annex 2. Test item accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 24 May 2022

Subject: **GMFF-22/01, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* bakery mix and rapeseed meal**

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 promptly inform us if this is not the case or if they arrived after 31 May 2022. There is no need to send proof of the delivery to the EURL GMFF.

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 for all issues related to this PT round.

Thank you for your collaboration.

Yours sincerely,

e-signed

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

Geel, 23 May 2022
JRC.F.5/WB/mt ARES(2022)3859264

«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/GMFF2201
Password	GMFF2201
Labcode	«LCode»

Subject: **Instructions for GMFF-22/01, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* bakery mix and rapeseed meal**

Dear Dr «Surname»,

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

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.

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 – Bakery 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 – Rapeseed meal (for feed):
- Verify the presence of GM oilseed rape in this sample;
- Quantify the GM event(s) identified and assess compliance of the sample.

Participants are requested to apply their routine approaches for GMO testing. 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.

The quantitative results have to be expressed in mass/mass % as outlined below and with a precision that you normally would use to report similar results (the value reported will be used to assess your performance score):

$$\text{mass/mass \%} = \frac{\text{mass GMO [g]}}{\text{total mass of the ingredient [g]}} \text{ x } 100$$

You are requested to pay attention to the correct estimation and reporting of the measurement uncertainty (to be expressed in m/m %, not as relative %) and coverage factor used. In addition to calculating your *z* scores, the uncertainties reported will be considered in the evaluation of the results using ζ (zeta) scores. Be aware of the existence of an appeal procedure in case you disagree with your scores.

You can find the MILC reporting website at https://web.jrc.ec.europa.eu/ilcReportingWeb. You need first to login with your EU login account (new procedure – see detailed guidelines) and then enter a personal password. Your unique password is indicated above in the box under your address data. The system will guide you through the reporting procedure.

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/GMFF2201, enter the password (see box above 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 before the reporting deadline (no need to send them by e-mail).

The deadline for the submission of the results ánd the questionnaire is Friday 8 July 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 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 MS8 oilseed rape in T2 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
19	0.57	0.6	0.57	0.55	0.49
23	0.57	0.55	0.59	0.53	0.58
40	0.56	0.55	0.57	0.52	0.55
46	0.54	0.54	0.5	0.52	0.53
79	0.53	0.56	0.52	0.55	0.52
83	0.53	0.58	0.46	0.6	0.57
102	0.55	0.53	0.55	0.55	0.56
Mean	0.55				
s_x	0.01				
s_w	0.03				
S_s	0				
u^*	0.01				
σ_{pt}	0.14				
$0.3 * \sigma_{pt}$	0.04				
$S_s \leq 0.3^* \sigma_{pt}$	0.01				
Assessment	Passed				

Homogeneity of RF3 oilseed rape in T2 (qPCR)

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
19	1.52	1.47	1.39	1.46	1.38
23	1.5	1.44	1.37	1.28	1.5
40	1.47	1.47	1.31	1.31	1.4
46	1.35	1.38	1.23	1.25	1.35
79	1.22	1.45	1.39	1.27	1.35
83	1.33	1.35	1.27	1.28	1.34
102	1.39	1.37	1.22	1.25	1.23
Mean	1.36				
s_x	0.06				
s_w	0.08				
S_s	0.05				
u^*	0.02				
σ_{pt}	0.30				
$0.3 * \sigma_{pt}$	0.09				
$S_s \leq 0.3^* \sigma_{pt}$	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 [17].

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 MS8 oilseed rape in T2 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	31	0.50	0.53	0.48	0.52
	105	0.49	0.56	0.57	
25	29	0.70	0.55	0.54	0.56
	98	0.52	0.47	0.59	

Slope ± 2 $SE_{(slope)}$ = 0.001 ± 2 * 0.001

Stability: passed

Stability RF3 oilseed rape in T2 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	31	1.54	1.40	1.10	1.33
	105	1.34	1.38	1.32	
25	29	1.32	1.25	1.34	1.30
	98	1.31	1.34	1.27	

Slope ± 2 $SE_{(slope)}$ = -0.001 ± 2 * 0.003

Stability: passed

Annex 5. Results and laboratory performance

ID = GM event identification (D = detected, ND = not detected, NT = not tested)
Compl. = Compliance statement (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.

GM event detection in T1

Lab code	Type	ID	Compl.
L01	NRL/625	ND	CNL
L02	NRL/625	ND	CNL
L03	NRL/120	ND	CNL
L04	OCL	ND	--
L05	NRL/625	ND	CNL
L06	NRL/120	ND	CNL
L07	NRL/120	ND	--
L08	NRL/625	ND	CNL
L09	NRL/625	ND	CNC
L10	NRL/625	ND	CNC
L11	OCL	ND	CNC
L12	OCL	ND	CNL
L13	NRL/120	ND	C<LLP
L14	NRL/625	ND	CNL
L15	NRL/625	ND	CNL
L16	NRL/625	ND	CNL
L17	OCL	ND	CNL
L18	NRL/625	ND	CNL
L19	NRL/120	ND	CNL
L20	OCL	ND	CNL
L21	NRL/120	NT	--
L22	NRL/625	ND	CNL
L23	NRL/625	ND	CNL
L24	NRL/625	ND	CNL
L25	NRL/625	ND	CNC
L26	NRL/625	ND	CNL
L27	NRL/120	ND	CNL, C<LLP
L28	NRL/625	ND	--
L29	NRL/625	ND	CNC
L30	NRL/625	ND	--
L31	NRL/625	ND	CNL
L32	NRL/120	ND	CNC

Lab code	Type	ID	Compl.
L33	OCL	ND	--
L34	NRL/120	ND	CNL
L35	NRL/120	ND	CNC
L36	NRL/625	ND	--
L38	NRL/625	ND	CNL
L39	NRL/625	ND	CNC
L40	NRL/625	ND	--
L41	OCL	ND	CNC
L42	NRL/120	ND	CNC
L43	OCL	ND	CNC
L44	NRL/625	ND	CNL, C<LLP
L45	NRL/625	ND	CNL
L46	NRL/120	ND	CNL
L47	NRL/625	ND	--
L48	NRL/120	ND	CNL
L49	NRL/625	ND	CNL
L50	OCL	ND	CNL
L51	OCL	ND	CNL
L52	NRL/120	ND	CNL, C<LLP
L53	NRL/625	ND	--
L54	NRL/625	ND	CNL
L55	NRL/120	ND	CNC
L56	NRL/120	ND	CNC
L57	OCL	ND	CNL
L58	NRL/120	ND	CNL
L59	NRL/625	ND	CNL, C<LLP
L60	NRL/625	ND	--
L61	NRL/120	ND	CNL
L62	NRL/625	ND	CNL
L63	NRL/625	ND	CNC
L64	NRL/625	ND	CNC

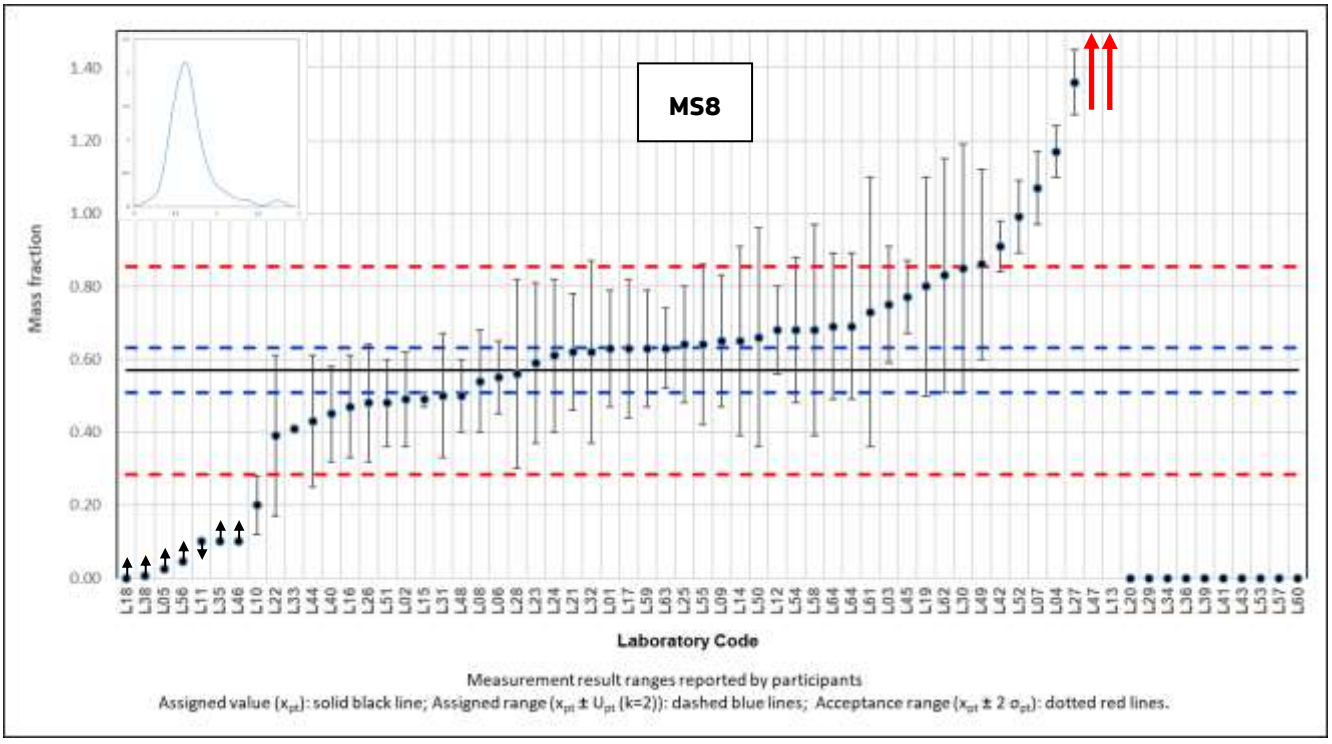
MS8 oilseed rape in T2

- ID = GM event identification (D = detected, ND = not detected, NT = not tested)
- "np" means that no measurement uncertainty was provided
- 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: $u(x_{pt,rel}) \leq u(x_i) \leq \sigma_{pt}$; b: $u(x_i) < u(x_{pt})$; c: $u(x_i) > \sigma_{pt}$; or np (not provided)
- Compliance (Compl.) statements (for the test item, not the individual GM event): see T1 above

Evaluation parameters: x_{pt} = 0.570 ; $u(x_{pt})$ = 0.031 ; σ_{pt} = 0.143 (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	0.63	0.16	2	Real-time PCR	0.42	0.70	a	NCL
L02	NRL/625	D	0.49	0.13	2	Real-time PCR	-0.56	-1.11	a	NCL
L03	NRL/120	D	0.75	0.16	2	Real-time PCR	1.26	2.10	a	NCL
L04	OCL	D	1.17	0.07	2		4.21	12.92	b	NCL
L05	NRL/625	D	> 0.025			Real-time PCR				CNC
L06	NRL/120	D	0.55	0.1	2	Real-time PCR	-0.14	-0.34	a	NC>LLP
L07	NRL/120	D	1.07	0.1	2	Real-time PCR	3.51	8.53	b	NCL, CNC
L08	NRL/625	D	0.54	0.14	2	Real-time PCR	-0.21	-0.39	a	NCL
L09	NRL/625	D	0.65	0.18	2	Real-time PCR	0.56	0.84	a	NCL
L10	NRL/625	D	0.2	0.08	2	Real-time PCR	-2.60	-7.35	a	CNL
L11	OCL	ND	< 0.1			Real-time PCR				CNC
L12	OCL	D	0.68	0.12	2		0.77	1.63	a	CNL
L13	NRL/120	D	4.36	2.82	2	Real-time PCR	26.60	2.69	c	NCL
L14	NRL/625	D	0.65	0.26	2	Real-time PCR	0.56	0.60	a	NCL
L15	NRL/625	D	0.49	0.02	2	Real-time PCR	-0.56	-2.49	b	NCL, NC>LLP
L16	NRL/625	D	0.47	0.14	2	Real-time PCR	-0.70	-1.31	a	NCL
L17	OCL	D	0.63	0.19	2	dPCR	0.42	0.60	a	NCL
L18	NRL/625	D	> 0							--
L19	NRL/120	D	0.8	0.3	2	Real-time PCR	1.61	1.50	a	NCL
L20	OCL	NT								CNC
L21	NRL/120	D	0.62	0.16	2	Real-time PCR	0.35	0.58	a	NCL
L22	NRL/625	D	0.39	0.22	2		-1.26	-1.58	c	NCL
L23	NRL/625	D	0.59	0.22	2	Real-time PCR	0.14	0.18	a	NCL
L24	NRL/625	D	0.61	0.21	2	Real-time PCR	0.28	0.37	a	NCL
L25	NRL/625	D	0.64	0.16	2	Real-time PCR	0.49	0.82	a	NCL
L26	NRL/625	D	0.48	0.16	2	Real-time PCR	-0.63	-1.05	a	NCL
L27	NRL/120	D	1.36	0.09	2	Real-time PCR	5.54	14.53	b	NCL, C<LLP
L28	NRL/625	D	0.56	0.26	2	Real-time PCR	-0.07	-0.08	a	NCL
L29	NRL/625	D								CNC
L30	NRL/625	D	0.85	0.34	2	Real-time PCR	1.96	1.62	a	CNL
L31	NRL/625	D	0.5	0.17	2	Real-time PCR	-0.49	-0.78	a	NCL, CNC
L32	NRL/120	D	0.62	0.25	2	Real-time PCR	0.35	0.39	a	NCL
L33	OCL	D	0.41	np		Real-time PCR	-1.12	-5.24	np	--
L34	NRL/120	D								CNC
L35	NRL/120	D	> 0.1			Real-time PCR				CNC
L36	NRL/625	D								--
L38	NRL/625	D	> 0.005			Real-time PCR				CNC
L39	NRL/625	D								CNC
L40	NRL/625	D	0.45	0.13	2	Real-time PCR	-0.84	-1.67	a	NCL
L41	OCL	NT								CNC
L42	NRL/120	D	0.91	0.07	3.18	dPCR	2.39	9.03	b	NCL
L43	OCL	D								CNC

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L44	NRL/625	D	0.43	0.18	2	Real-time PCR	-0.98	-1.47	a	NCL
L45	NRL/625	D	0.77	0.1	2	Real-time PCR	1.40	3.41	a	NCL
L46	NRL/120	D	> 0.1			Conv. PCR+gel				CNC
L47	NRL/625	D	1.73	0.156	2	Real-time PCR	8.14	13.85	b	CNC
L48	NRL/120	D	0.5	0.1	2	Real-time PCR	-0.49	-1.20	a	CNC
L49	NRL/625	D	0.86	0.26	2	Real-time PCR	2.03	2.17	a	CNL
L50	OCL	D	0.66	0.3	2	Real-time PCR	0.63	0.59	a	NCL
L51	OCL	D	0.48	0.12	2	Real-time PCR	-0.63	-1.34	a	NCL
L52	NRL/120	D	0.99	0.1	2	Real-time PCR	2.95	7.17	b	NCL, C<LLP
L53	NRL/625	ND								--
L54	NRL/625	D	0.68	0.2	2	Real-time PCR	0.77	1.05	a	NCL
L55	NRL/120	D	0.64	0.22	2	Real-time PCR	0.49	0.61	a	NCL
L56	NRL/120	D	> 0.045			Real-time PCR				CNC
L57	OCL	D								CNL
L58	NRL/120	D	0.68	0.29	2.228	Real-time PCR	0.77	0.82	a	NCL, NC>LLP
L59	NRL/625	D	0.63	0.16	2	Real-time PCR	0.42	0.70	a	NCL, C<LLP
L60	NRL/625	ND								--
L61	NRL/120	D	0.73	0.37	2	dPCR	1.12	0.85	c	CNL
L62	NRL/625	D	0.83	0.32	2	Real-time PCR	1.82	1.60	a	CNL
L63	NRL/625	D	0.63	0.11	2	Real-time PCR	0.42	0.95	a	NCL
L64	NRL/625	D	0.69	0.2	2	Real-time PCR	0.84	1.15	a	CNC



Note: The values reported by L13 and L47 are out of scale and therefore not visible on the graph (red arrows).
Upper left: kernel density distribution

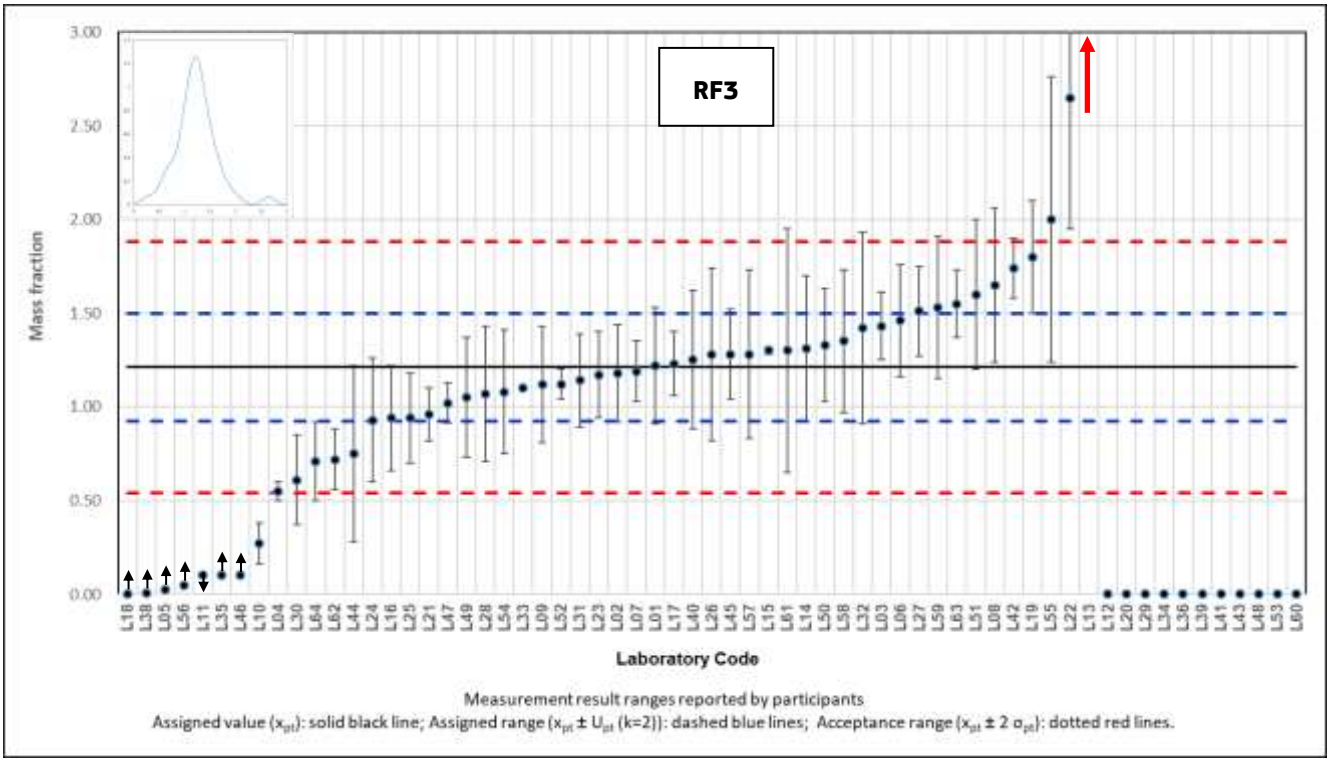
RF3 oilseed rape in T2

- ID = GM event identification (D = detected, ND = not detected, NT = not tested)
- "np" means that no measurement uncertainty was provided
- 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: $u(x_{pt,rel}) \leq u(x_i) \leq \sigma_{pt}$; b: $u(x_i) < u(x_{pt})$; c: $u(x_i) > \sigma_{pt}$; or np (not provided)
- Compliance (Compl.) statements (for the test item, not the individual GM event): see T1 above

Evaluation parameters: x_{pt} = 1.213 ; $u(x_{pt})$ = 0.137 ; σ_{pt} = 0.303 (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	1.22	0.31	2	Real-time PCR	0.02	0.04	a	NCL
L02	NRL/625	D	1.18	0.26	2	Real-time PCR	-0.10	-0.17	b	NCL
L03	NRL/120	D	1.43	0.18	2	Real-time PCR	0.65	1.28	b	NCL
L04	OCL	D	0.55	0.05	2		-1.98	-4.54	b	NCL
L05	NRL/625	D	> 0.025			Real-time PCR				CNC
L06	NRL/120	D	1.46	0.3	2	Real-time PCR	0.74	1.19	b	NC>LLP
L07	NRL/120	D	1.19	0.16	2	Real-time PCR	-0.07	-0.14	b	NCL, CNC
L08	NRL/625	D	1.65	0.41	2	Real-time PCR	1.30	1.75	a	NCL
L09	NRL/625	D	1.12	0.31	2	Real-time PCR	-0.28	-0.44	a	NCL
L10	NRL/625	D	0.27	0.11	2	Real-time PCR	-2.81	-6.13	a	CNL
L11	OCL	ND	< 0.1			Real-time PCR				CNC
L12	OCL	NT								CNL
L13	NRL/120	D	9.45	6.04	2	Real-time PCR	24.56	2.72	c	NCL
L14	NRL/625	D	1.31	0.39	2	Real-time PCR	0.29	0.40	a	NCL
L15	NRL/625	D	1.3	0.02	2	Real-time PCR	0.26	0.61	b	NCL, NC>LLP
L16	NRL/625	D	0.94	0.28	2	Real-time PCR	-0.81	-1.36	a	NCL
L17	OCL	D	1.23	0.17	2	dPCR	0.05	0.10	b	NCL
L18	NRL/625	D	> 0							--
L19	NRL/120	D	1.8	0.3	2	Real-time PCR	1.75	2.83	b	NCL
L20	OCL	NT								CNC
L21	NRL/120	D	0.96	0.14	2	Real-time PCR	-0.75	-1.58	b	NCL
L22	NRL/625	D	2.65	0.7	2		4.28	3.80	a	NCL
L23	NRL/625	D	1.17	0.23	2	Real-time PCR	-0.13	-0.23	b	NCL
L24	NRL/625	D	0.93	0.33	2	Real-time PCR	-0.84	-1.29	a	NCL
L25	NRL/625	D	0.94	0.24	2	Real-time PCR	-0.81	-1.46	a	NCL
L26	NRL/625	D	1.28	0.46	2	Real-time PCR	0.20	0.25	a	NCL
L27	NRL/120	D	1.51	0.24	2	Real-time PCR	0.89	1.59	b	NCL, C<LLP
L28	NRL/625	D	1.07	0.36	2	Real-time PCR	-0.43	-0.62	a	NCL
L29	NRL/625	D								CNC
L30	NRL/625	D	0.61	0.24	2	Real-time PCR	-1.80	-3.22	a	CNL
L31	NRL/625	D	1.14	0.25	2	Real-time PCR	-0.22	-0.38	b	NCL, CNC
L32	NRL/120	D	1.42	0.51	2	Real-time PCR	0.62	0.71	a	NCL
L33	OCL	D	1.1	np		Real-time PCR	-0.34	-0.78	np	--
L34	NRL/120	D								CNC
L35	NRL/120	D	> 0.1			Real-time PCR				CNC
L36	NRL/625	D								--
L38	NRL/625	D	> 0.005			Real-time PCR				CNC
L39	NRL/625	D								CNC
L40	NRL/625	D	1.25	0.37	2	Real-time PCR	0.11	0.16	a	NCL
L41	OCL	NT								CNC
L42	NRL/120	D	1.74	0.16	3.18	dPCR	1.57	3.46	b	NCL
L43	OCL	D								CNC

Labcode	Type	ID	x_i	$U(x_i)$	k	Technique	z score	ζ score	MU	Compl.
L44	NRL/625	D	0.75	0.47	2	Real-time PCR	-1.38	-1.68	c	NCL
L45	NRL/625	D	1.28	0.24	2	dPCR	0.20	0.36	b	NCL
L46	NRL/120	D	> 0.1			Conv. PCR+gel				CNC
L47	NRL/625	D	1.02	0.108	2	Real-time PCR	-0.57	-1.25	b	CNC
L48	NRL/120	D								CNC
L49	NRL/625	D	1.05	0.32	2	Real-time PCR	-0.48	-0.76	a	CNL
L50	OCL	D	1.33	0.3	2	Real-time PCR	0.35	0.57	b	NCL
L51	OCL	D	1.6	0.4	2	Real-time PCR	1.15	1.57	a	NCL
L52	NRL/120	D	1.12	0.08	2	Real-time PCR	-0.28	-0.62	b	NCL, C<LLP
L53	NRL/625	ND								--
L54	NRL/625	D	1.08	0.33	2	Real-time PCR	-0.40	-0.61	a	NCL
L55	NRL/120	D	2.00	0.76	2	Real-time PCR	2.35	1.94	a	NCL
L56	NRL/120	D	> 0.045			Real-time PCR				CNC
L57	OCL	D	1.28	0.45	2	Real-time PCR	0.20	0.25	a	CNL
L58	NRL/120	D	1.35	0.38	2.262	Real-time PCR	0.41	0.62	a	NCL, NC>LLP
L59	NRL/625	D	1.53	0.38	2	Real-time PCR	0.95	1.33	a	NCL, C<LLP
L60	NRL/625	ND								--
L61	NRL/120	D	1.3	0.65	2	dPCR	0.26	0.25	a	CNL
L62	NRL/625	D	0.72	0.16	2	Real-time PCR	-1.47	-3.00	b	CNL
L63	NRL/625	D	1.55	0.18	2	Real-time PCR	1.01	1.99	b	NCL
L64	NRL/625	D	0.71	0.21	2	Real-time PCR	-1.50	-2.82	a	CNC



Note: The value reported by L13 is out of scale and therefore not visible on the graph (red arrow).
Upper left: 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	1*	1*
No Answer	0	0

* Reason: The sample matrix is out of the scope of our laboratory

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

	T1	Ratio	T2	Ratio
Yes	44	69.84%	39	61.9%
No	3	4.76%	6	9.52%
Partially	15	23.81%	18	28.57%
Not applicable	1	1.59%	0	0%
No Answer	0	0%	0	0%

Further explanations

Not yet flexible scope
Matrix feed
Maize MON810, GA21, MIR162, MIR604, MON87460, MON88017, MON89034 are not within the scope Rapeseed MON88302, MS8, RF3 and T45 are not within the scope.
The lab is accredited under the flexible scope. Events MS8 and RF3 were for the first time used, therefore they were not yet reported to the Accreditation Body (IPAC).
Not all events are accredited
T1 - food matrix is not within the scope of our accreditation.
Ms8 and Rf3 quantitative methods are not accredited in our lab
All used screening methods are under scope of accreditation and all used event specific methods are not accredited.
The method is being validated and accredited
Some methods used to detect and quantify rapeseed events are not yet verified in our lab.
Our scope implements the DNA-analysis per se, especially in the matrices feed and seed.
Feed (T2) is not under accreditation, Ms8 and Rf3 are not quantitatively verified. MON88302, 73496 are not verified at all. Some events from T1 and T2 are excluded by negative screening results.
Some methods are not yet verified in the lab
Some methods not accredited but in verification

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	17	2	4	43	2	1	0
T2	13	1	3	42	3	1	0

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	20	18
CTAB + Maxwell 16 Food, Feed, Seed	6	7
NucleoSpin Food	18	6
NucleoSpin Plant	2	2
GeneSpin	5	4
Promega Wizard	3	2
Qiagen DNeasy Plant	1	2
Qiagen DNeasy Mericon Food	5	3
Biotecon Foodproof	4	3
SDS	2	1
Fast ID Genomic DNA	0	0
Generon Ion Force	0	0
Eurofins DNAExtractor cleaning column	2	2
Promega Wizard DNA clean-up resin	1	2
Qiagen QIAQuick	2	1
Qiagen Genomic-Tip 20/G	0	0
NucleoSpin gDNA clean-up	0	0
Other	11	12

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

Before weighing test portions for DNA extraction (4 x 200 mg), the samples were milled with a Retsch MM400 small ball mill, in order to obtain an extra fine powder
"CTAB-precipitation" method
T1 and T2 after extraction, purification with QIAquick (R) PCR purification kit
Reference material for quantification was provided from AOCS as DNA-solution: --> no extraction possible
Extra chloroform step (2x) was used to eliminate the PCR inhibitors from the sample (T2).
Sample intake: 500 mg. The volume of lysis buffer CF and proteinase K solution were increased by factor 2.5x
The CTAB method used for the maize-based matrix is different for the one used for rapeseed meal. The latter is based on the CRLVL26/04XP method. DNA extracts from rapeseed meal were purified using "DNA clean & concentrator -25 (ZymoResearch, Cat. No. D4006).
InnuPrep DNA extraction Kit for Innupre C16 from Analytic Jena
CTAB + Maxwell RSC PureFood GMO and Authentication Kit
Very small amount of extracted DNA in T1 sample.
Modified Qiagen Blood & Tissue kit
Used Kit: NucleoMag Food (Macherey Nagel)
For T1 Maxwell DNA isolation performed as normal and in a second attempt in combination with alfa-amylase to get rid of carbohydrates
T2: Quick-DNA Plant/Seed Miniprep Kit
Lysis in CTAB buffer with Proteinase K and RNAse, clean up of supernatant with NucleoMag Plant
EURx GeneMatrix Food_Extract DNA Purification Kit
SpeedTools Food DNA Extraction Kit (Biotools)
T1: after SDS: Maxwell RSC pureFood GMO and Authentication Kit
T2: CTAB/Wizard according to SLMB, additional clean-up by MicroSpin S-300 HR column
T1: SureFood®Prep Advanced Kit
Maxwell RSC PureFood GMO and Authentication Kit
Overnight incubation, 2x washing CQW buffer for T1 sample

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

Answer	**T1**	**T2**
No	5	5
We performed a PCR inhibition test on a reference gene target prior to the analysis	29	28
We performed a PCR inhibition test on a GM gene target prior to the analysis	4	3
We analysed two or more dilutions of the DNA and compared the results	32	34
An internal positive control was added to the unknown samples	9	9
Other	3	3

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

Spectrophotometric measurement of concentration and OD ratios: 12 answers
Different dilutions of the DNA extract were analysed, quantification of the reference gene did indicate inhibition
One of four tested extracts for T1 gave inhibition in the undiluted extract and was discarded before further PCR analyses. No inhibition seen in 3/4 T1 extracts nor 4/4 T2 extracts.
DNA quality check: ratio of absorbance and in the course of the PCR inhibition controls and at least in two different DNA concentrations (if possible with 40 µg/µL and diluted 1:4). Different DNA extraction methods were used for the proficiency test. Apart from a low yield of amplifiable maize DNA in T1, no inhibition could be detected for either sample.
Reference gene is analysed in two different dilutions and the resulting dCT is compared to the expected dCT
2 dilutions of the endogenous reference sequence in screening, 3 dilutions of the DNA in quantification
Besides the dilutions we also used a positive control, a negative control and one environmental control
Because T2 was provided as powder, no testing for zygosity was possible.
Amplificación de gen endógeno
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: *CruA*). The delta Cq-value was assessed for PCR inhibition (expected delta Cq +/-0.5). For T1 a slight inhibition was observed (within the tolerance), T2 was not inhibited.
We made a dilution 1:10 of the sample and we add p35s on both samples (diluted and non diluted). We obtained the same Ct value on both samples (36.74 Vs 36.56). We measured DNA with a nanodrop and the ratio A260/280 and A230/260 were both over 1.7.
Fluorometric quantification. Inhibition assumed: T1: amplification-control with endpoint-PCR only in high dilution positive, realtime PCR: ct (*hmg*) 32,6 at 60 ng DNA per reaction
No inhibition was detected: 2 answers
In addition to inhibition testing, fragmentation of DNA was analyzed by Capillary electrophoresis. No severe fragmentation was found.
1. CTAB extraction: Inhibition test based on reference gene system (*HMG*) was performed according to guidelines in JRC Technical Report "Verification of anal. Meth. for GMO testing when Implem. Interlab. Validated Methods", 2017, Annex 2 Working DNA quantity 100ng DNA /reaction. RESULT: No inhibition. Working DNA concentration 200ng DNA /reaction. RESULT: Inhibition. After treatment of DNA with Microcon columns (Millipore), an inhibition test based on *HMG* was performed. Working DNA quantity 200ng DNA /reaction. RESULT: No Inhibition. 2. Nucleospin extraction: Inhibition test based on *HMG* was performed according to the above-mentioned Report. Working DNA concentration 200ng DNA /reaction. RESULT: Inhibition. After treatment of DNA with Microcon columns (Millipore), an inhibition test based on *HMG* was performed Working DNA quantity 200ng DNA /reaction. RESULT: Inhibition
We examine the curves for the reference gene
Positive and negative control in each run.

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

Screening target	T1: present	T1: absent	T2: present	T2: absent
P35S	12	45	42	9
tNOS	12	43	53	0
PAT	1	43	2	40
BAR	0	41	41	0
CP4-EPSPS	0	9	5	2
Ctp-CP4-EPSPS	0	0	1	0
Ctp2-CP4-EPSPS	7	30	33	4
Cry1Ab/Ac	3	15	9	2
Cry1Ab	0	4	3	0
pFMV	3	18	18	3
pNOS	0	7	1	7
t35S	0	2	0	1
nptII	0	10	1	9
p35S-pat	0	9	0	10
tE9	5	10	15	2
Other	2	9	8	8

Further details on other screening targets

T1: 2 out of 3 tested/useful (i.e. no inhibition) extracts give positive signal for pFMV. All maize events carrying pFMV were therefore further assessed and found negative.
Positive in T1 and T2: AgroBorder II (sequence from Agrobacterium tumefaciens Ti-plasmid of octopine type, used as construct-flanking sequence in different gm plant, e.g. MONxx; Negative: construct of cassava Vein Mosaic Virus Promoter (P-CsVMV) and pat from S. viridochromogenes. Negative: AgroBorder I (nos -promoter-sequence from A. tumefaciens Ti-plasmides of nopalin-type. The 3 screening sequences will soon be included into national standard collection in Germany.
P-nos-nptII in T1 and T2 absent; CaMV in T2 present
T1: Event-specific real-time PCR DAS40278/VCO-01981-5/MON87419: < LOD (0.3 m/m); T2: CaMV present
To identify the GMO in T1, a screening was done using GM maize event-specific tetraplex and singleplex qPCR assays. To identify the GMO in T2, a screening was done using GM oilseed rape event-specific tetraplex and singleplex qPCR assays.
The above 8 screening elements are the ones implemented in the lab. As they do not allow for detecting all maize events, additionally, DAS40278, VCO-01981 and LY038 were used for T1.
We screened the samples in usual method - and with T2 also CaMV which was positive
pSSUAra/Bar was positive in T2
For T1: the Ct values of p35S and tNOS are above 40
T1: Event-specific methods LY038, DAS-40278, VCO-01981 and MON87419 (all absent) T2: p35S-nptII (absent), DP-073496 (absent), GT73 (absent), MON88302 (absent), OXY235 (absent), T2: Lectin (present), MON89788 (present), MON87705 (absent)
T1 sample: *hmg*, *CruA*, lec, PLD and SAD were detected; T2 sample: *CruA* and lec were detected.
T1: For Cry1Ab/Ac and tE9 the individual Cq-values were 37.10 and 38.48. T2: The Cq-values for p35S were around 36.10 and were experimentally confirmed to derive from the natural source of CaMV.
We detected screening targets when we loaded higher DNA concentration (other than method recommended) for T1.
T2: p35S-nptII
DAS40278 was also tested, but not detected.
T1 additionally tested for MON863, MON87460, MIR604, GA21, MIR162, DAS40278, 5307, VCO1981, MON87403, 3272. T2 additionally tested for GT73, 88302 and 73469
T1: present: AgroBorder Sequence II T2: present: bar-Tg7; AgroborderSequence II, CaMV; absent: p35S-nptII T1+T2 absent: ABS I; PCsVMV-pat
T1: rice actin absent; p35S: maxwell isolation absent, CTAB isolation present T2: gat/T-pinII absent p35S and pFMV found present and not explained by MS8 and Rf3, this is an indication that there is more GMO present in the sample.
Triplex-Method including elements for AgroBorder I and AgroBorder II and P-CsVMV-pat-construct
T2: CAMV
We also consider as screening targets those events that have no "regulatory" elements or constructs. In T1 DAS40278 and VCO1981 were analysed and they were not detected. We also detected lectin reference gene so events 305423, CV127, MON87701 and MON87751 were analysed and they were not detected. In T2 event 73496 was analysed and not detected.
T2 - we have found taxon for maize - *hmg*; Soybean - Lec; Oilseed rape *CruA*; Potato - OPG
T2: p35S seems to be present, but cauliflower mosaic virus was also positive (often in oilseed rape as virus disease); Positive CTP2-CP4-EPSPS and pFMV could not be explained by the six events to be reported, perhaps botany contamination by MON89788 soy.
PCR for PAT gene and Cry1Ab/Ac gene gave ambiguous results.
For T1 the p35S is very weak with Cq signals from 36 to no Cq

Which GM event was identified in T1 and T2? Note that your answer to this question will be used to assess your lab performance for GM event identification.

See this report.

Do you consider the DNA extracted from T1 and T2 as suitable for quantitative PCR analyses? Did you see any indications that this is not the case?

Yes, we consider the DNA extracted for T1 and T2 suitable for quantitative PCR: 23 answers
T1 yes; T2 no: 2 answers
T1: Much DNA extracted (measured fluorometrically), however only few amplifiable maize DNA, therefore high practical LOD, if only 200 ng DNA is used for PCR)
Despite the fact that we did not observe inhibition in the PCR (not with endogenous ref gene nor with MS8 & RF3 GMO targets), the undiluted, pure T2 DNA extracts did not deliver useful results in the absolute quantification qPCRs for both MS8 and RF3 (Cts of both species- and GM-specific targets were too high and out of standard curve range). So quantitative data are based on the 1:4 DNA extracts for T2.
I consider the DNA extracted from T2 as suitable for quantitative PCR analyses. In T1 we extracted very small quantity maize (Ct for *hmg* gene is 29-33) which can be insufficient for identificated GM event.
From T1, DNA extracted is not suitable for quantitative PCR.
T1: Quantification limited to GM contents > 1 m/m %, practical LOD: 0.3 m/m %, practical LOQ: about 1 m/m %; T2: quantification without limitations, practical LOD: 0.1 m/m %
T1: No quantification was performed for T1. The content of amplifiable DNA or the obtained Cq value for the maize reference could be a challenge for a reliable quantification of GMO contents in the range of the limit of quantification of 0.1 %. The sample material would be suitable for checking the labelling threshold. T2: suitable
For both samples we measured high DNA concentrations (T1: 700ng/ul; T2: >1000ng/ul). For T1 only a small amount comes from maize (high Cq-value of *hmg* detection). This could influence the sensitivity for GM maize events (practical LOQ or LOD).
Yes. All DNA extracts were diluted to 20 ng/uL. Amplificability was checked by amplifying *hmg* and FatA endogenous genes, respectively. Ct values for *hmg* were higher than usual (approx. 28). Therefore, screening was done with 3 uL of extracts diluted to 20 ng/uL (as in routine), 50 ng/uL and 100 ng/uL (to ensure detection).
Sample T1 showed some inhibition
DNA extracted from T1 and T2 were suitable for quantitative PCR analyses. In the case of sample T1, although we did not identify any GM event of maize, we did identify the presence of reference genes: *HMG*, lec, *CruA* and PLD. We used PSP plates for screening and identification of GM maize in sample T1 and PSP plates for screening in sample T2.
Sample T1 was not suitable for quantification, pLOD for sample T1 was >0,9%
For sample T1 (bakery dry mix) the extracted amount of target taxon DNA (maize) was so low (~ 1.2 ng / µl) that this amount is insufficient for the quantification of GM maize around the legal threshold of 0.9 % due to a practical LOQ which is way above that threshold. Example: With an assumed absolute LOQ of a method of 40 cp and with 2200 cp reference genes (*hmg*) per PCR reaction this would lead to a practical LOQ of around 3.3 % (with heterozygous GM maize). For sample T2 (rapeseed meal) the DNA extracted was suitable for quantitative PCR analysis as the extractable amount of target taxon DNA (canola) was sufficient to achieve a practical LOQ of around 0.1 % and consequently was sufficient to quantify around the legal threshold of 0.9 %. Note: Percentage values are given in m/m %. The amount (concentration) of extracted TARGET taxon (maize) DNA was determined by ddPCR.
Yes for T2. However, we do not have quantitative methods for MS8 and Rf3. In T1, quite few *HMG*-copies were detected and none of the indicated screening methods below were detected.
We only performed qualitative analyses.
For T1 consider that DNA extracted is not suitable for quantitative PCR analyses. I had no amplifications neither in screening test nor in identifying events and Ct values for reference gene it was between 27-31
Inhibition assumed: T2: quantification in strong dilutions (180, 90, 45, 22 ng)
After many dilutions of DNA, we got enough DNA extraction for quantitative PCR analyses
There are indications that the DNA extracted from T1 may not be suitable for quantitative PCR analyses. Working with the usual DNA quantity (100 ng/reaction) of CTAB DNA extracts, we have not observed any inhibition in real time PCR for *HMG* gene, but we have not obtained any positive signal for screening elements (p35s, tNOS, pat, CryIAb/Ac). By increasing the DNA concentration, positive signals for p35s and tNOS were observed. However, we had indications for inhibition (as shown by PCR for Internal Positive Control) at this DNA concentration. Nevertheless, real time PCR runs for the identification of GM events (NK603, TC 1507, Bt11, DAS-40278, MON810, MIR 604 and DAS 59122), using diluted (100 or 200 ng/reaction) and undiluted DNA extracts, showed negative results.
Yes. T1 had a ct value of 27 for the reference gene which indicates that the maize content is low.
We observed late amplification for reference gene in T1 sample (29-30 ct)

Which quantification approach was used?

Quantification approach	**T1**	**T2**
Standard curve method (2 calibration curves)	11	41
Delta Cq method (one calibration curve)	0	4
Digital PCR	0	4
No quantification done	44	14

Which master mix was used for T1 and T2 analysis?

Master Mix	**Answers**
2x TaqMan Universal PCR Master Mix (Applied Biosystems-Thermofisher)	34
ddPCR Supermix for Probes no dUTP (Bio-Rad)	4
Qiagen Quantitect Multiplex no ROX	4
Maxima Probe qPCR Master Mix	3
EUROGENTEC qPCR Mastermix Plus without UNG	3
2x GoTaq Probe qPCR MasterMix (Promega)	3
Sigma Jumpstart Ready mix	2
PerfeCTaqPCR ToughMix or Fast Mix (Quantabio)	2
Taqman environmental mastermix 2.0	1

Quantinova Qiagen	1
Kapa Probe Fast qPCR Master Mix (2X)	1
IQSupermix (BioRad)	1
Brilliant III Ultra-Fast qPCR Master Mix (Agilent, Art.no.600881)	1
LightCycler FastStart DNA HybProbe (Roche)	1
Qiagen HotStarTaq	1
LUNA Universal Probe qPCR MasterMix - New England Biolabs	1
Brilliant-Agilent	1
Eagle Taq Universal Master Mix (Roche)	1

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

Target	CRM code	Answers
MS8	0306-F	2
	0306-F2	3
	0306-F3	10
	0306-F4	1
	0306-F5	2
	0306-F6	2
	0306-F7	7
	0306-F8	18
RF3	0306-G	4
	0306-G2	3
	0306-G3	5
	0306-G5	4
	0306-G6	10
	0306-G7	18

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

Target	Average	Minimum	Maximum
MS8	-3.40	-3.67	-3.10
RF3	-3.45	-3.68	-3.20
Taxon	-3.48	-3.80	-3.25

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

Test item	Reference target	Answers	Ratio
T2	*CruA*	37	67.27%
	Ccf	1	1.82%
	FatA(A)	10	18.18%
	Other	7	12.73%

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?	Reference target	Conversion factor
/	Other (*PepC*)	RF3 Homozygous; MS8 Hemizygous; Quantification, calculation, CF: done by BfUL Nossen
/	Other (*PepC*)	RF3: 0.907 ; MS8: 0.456 and MON89788: 0.981
dPCR	*CruA*	Ms8: 0,46 (homozygote, but 2 copies of *CruA*); Rf3: 0,23 (hemizygote, 2 copies of *CruA*)
/	*CruA*	T2: MS8 CF=0.456; RF3 CF=0.907
dPCR	*FatA(A)*	T2: MS8 (AOCS 0306-F7): 0.483 +/- 0.017; RF3 (AOCS 0306-G6): 0.920 +/- 0.011
dPCR	*FatA(A)*	T2: CF for Rf3 (0306-G6): 0.907
dPCR	*FatA(A)*	For T2: GM MS8 Conversion factor 0.456 was used. For T2: GM RF3 Conversion factor 0.907 was used.

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?

Compliance statement	Code	T1	T2
Compliant to Regulation 1829/2003 because no labelling required (present at </= 0.9 m/m % if adventitious or technically unavoidable)	CNL	36	7
Not compliant to Regulation 1829/2003 (present at >0.9 m/m %, hence requiring labelling)	NCL	0	34
Compliant to Regulation 619/2011 (present in feed at <0.1 m/m % if adventitious or technically unavoidable)	C<LLP	5	3
Not compliant to Regulation 619/2011	C>LLP	0	3
Cannot be concluded	CNC	15	18

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

T1 : No event identified > LOD or labelling threshold ; T2 : GM%MS8 + GM%RF3 - MU (MS8+RF3) > 0.9%
Amount (under repeatability conditions) above the limit using the authorized CRM; Considering the uncertainty of EURL-method validation (RSD_R=23%) the amount as stacked event spans the limit with (n=3 extracts) --> investigation of further extracts would be necessary to lower the uncertainty. Mismatches in primers and probe annealing region in the official *CruA*-method, uncertainty due to different extraction of CRM and sample, different tissue leaf/seeds and unknown zygosity of sample T2 have to be emphasized!
T1 is consider compliant because no GM event was detected. T2 is consider not compliant because the sum of the detected percentages of the two GM events is >0.9 m/m %

T1: Nothing found, so it must comply. T2: If the signal from RF3 and MS8 originates from a stacked event, the results comply to regulation 1829/2003. If the signals from RF3 and MS8 originate from two single events, the result is 1.55 +/- 0.39 w/w and should be labelled. Our CA would evaluate the result as it probably resulted from the stack and therefore compliant with the legislation
T2: Ms8 and Rf3 are authorised GM rapeseed events in the EU, hence the labelling threshold to be applied is 0.9 m/m%. Our results were: Rf3= 1,31%, Ms8= 0,65%, together is=1,96-MU > 0.9 m/m %. So T2 is not compliant, should have been labelled.
T1: The sample complies with the EU GMO legislation, no GM maize events could be identified. Traces of the approved GM soy events MON40-3-2 and MON87701 have no impact on the result. Food inspectors should inspect the farm/manufacturer to check formulations and, if necessary, sample starting materials and have them tested for GM events. T2: MS8, RF3, MS8xRF3 is authorized for the use in food/feed within EU. The competent authority in Austria follows the addition approach. The result for MS8 and RF3 after addition and including the measurement uncertainty is 1.41% +/- 0.42%. The lower 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.
Test item T2: The Rf3 and Ms8 are an authorised feed product in the EU, hence: - the labelling threshold to be applied is 0,9 m/m% - Knowing that the assigned range is: Ms8: 0,65±0,18 (k=2) m/m%, Rf3: 1,12±0,31 (k=2) m/m%, total GM: 1,77±0,36 (k=2) m/m%. - This material is to be considered as "Not compliant to Regulation 1829/2003" (1,77-0,36 >0.9 m/m % - requiring labelling)
Ms8&Rf3 are authorised (renewal ongoing). The total amount of GM oilseed rape events is 3.04% >0.9%. Remark: Ms8xRf3 is a stacked event, however our CA require that the sum of all events is made in order to assess the compliance.
T1 - We did not identify any GM event of maize in sample T1, therefore the sample is compliant with the EU GMO legislation. T2 - During the detection and identification of GMO in sample T2 we detected presence of two authorised GM events of oilseed rape: Ms8 and Rf3. From our measurement results (sum of both results) and after the subtraction of combined uncertainty from these sum, the lowest value of our result (result ± combined uncertainty) is above 0.9% - it means, that the sample is not compliant with the EU GMO legislation and labelling is required. In this case we assume that the sample contains two single events of oilseed rape and therefore is not compliant with the EU GMO legislation and labelling is required. NOTE: If we had documentation that the sample contained Two -Event Stack oilseed rape Ms8xRf3 (and CRM - Ms8xRf3 was available), then the sample would be in compliance with the EU GMO legislation and it would not require labelling
As the stacked event canot be differentiated from the two single events, no conclusion can be drawn as a) if the two single events are present the total gm-content would amount to 1.58 % +/- 0.21 %, therefore: not compliant to Reg 1829/2003 (labelling required); b) stacked event present (app. 1,0 +/- 0,15), it would be an unsure deviation of the 0,9 % border, therefore compliant to reg 1829/2003 (no labelling required if adventitious or techn. unavoidable)
T1: This sample (bakery mix) is an unknown mixture of ingredients containing maize. To judge the legal compliance (Q. 1) of the product concerning the ingredient maize an appropriate PRACTICAL LOD and LOQ is necessary to reliably detect and quantify GM DNA around the legal threshold of 0.9 % (m/m). The amount of extractable target taxon DNA (maize) was very low (1.2 ng/µl). Consequently the practical LOD is 0.91 % (m/m) and the practical LOQ is 3.3 % (m/m), assuming an absolute LOD of 10 cp and an absolute LOQ of 40 cp for heterozygous GM maize. Therefore the legal compliance of the sample cannot be concluded. T2: This sample (feed) is 100 % rapeseed meal. Two GM canola events (MS8 and RF3) were detected and quantified. From the quantification result of each event the individual extended MU (U) were substracted and the resulting GMO contents (m/m %) were summed up. The result is 1.28 % (m/m) which is above the legal threshold of 0.9 % (m/m), hence labelling is required.
T2: according to the competent authority in Austria the quantitative results of the same taxa are added up. The result for MS8 and RF3 after addition taking into account the measurement uncertainty is 1.70% ± 0.51%. As the lower value of 1.19% is above the labelling threshold of 0.9% the sample is considered not compliant with the legal requirements, it would have to be labelled as GMO.

Additional comments and suggestions (only the most informative answers are shown).

As no maize event (listed in the reporting page) was identified in T1, a screening approach was used in parallel. Amplifications plots were observed for p35S, tNos, pFMV and CTP2-CP4-EPSPS. Even if these amplification plots were considered as below LOD of respective methods, identification tests (other than the ones requested) were also performed on T1. Amplification plots (also considered as below LOD) were observed for MON 89788 and GTS 40-3-2 soybean, that can explain amplification plots observed for the screening elements (MON 89788 for pFMV and CTP2-CP4-EPSPS; GTS40-3-2 for p35SQ and tNos).
T1: only few maize DNA amplifiable, practical LOD 0,2 %, if around 240 ng sample DNA is used
We identificated two soybean GM events in Test item 2 in quantity: 23,24±6,51 (k=2) m/m% MON89788 and 51,30±14,36 (k=2) m/m% MON87701. Code of the CRM used for quantification: AOCS0906-B2, AOCS0906-A2, AOCS0809-A Slope of calibration curves for GM and taxon targets in T2: MON89788 soybean: MON89788 -3,479, lec -3,24; MON87701 soybean: MON87701 -3,457, lec -3,555
T1: by applying our screening approach for GM maize events using 100ng/PCR genomic DNA, we could not detect any GM maize events in the sample. By increasing to 300ng/PCR we detected weak amplification signals for T-NOS, CryAb/Ac and EPSPS (<LOQ). T2: in GM-event screening we could also detect traces of T45, but the quantification using ddPCR was not successful (below LOQ).
T1 sample was somehow off - even though several DNA concentrations were tested because of inhibition, still NO GM events could be identified. The initially observed T-NOS and EPSPS signals were also very weak. Our practical LOD for T1 was also a bit high (0.56) and LOQ was 1.4 (affected by low sample average Starting quantity of 2603 gene copies). Was our sample degrated and thus escaped identification? DNA concentrations were fine when measured with dsDNA RB Qubit-kit, and we used 100ng/DNA per reaction.
In the sample T2 we found GM Soybean Event MON89788 with an amount 33,9% +/- 7,5% (m/m).
Although we had 16 out of 23 GM events for sample one and did not find any, it might be a good idea to list 3-4 GM events in PT instructions what we are looking for so that we can order standards and reagents in time.
T 1: DNA Isolation was repeated using 1 g sample intake applying CTAB method with 2 % CTAB in lysis buffer. The DNA concentration was almost identical compared to the previously applied 200 mg sample intake and CTAB + Maxwell 16 Food, Feed, Seed method.
The contamination of T2 with MON89788 soybean and of T1 with probably GM soybean or with the material of T2 made this ring trial far more time-consuming than necessary. Since T1 was positive for rapeseed and T2 positive for maize: could a cross-contamination of both samples be excluded?
We were not able to identify any GMO for T1, despite many purifications and runs. Primary screenings, but also specific PCR for events which should be positive for p35S. With a Ct value of the reference gene of 27, we expect that we would have identified events above the 0.9 % content.

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