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Determination of GM Oilseed Rape MON88302 in rapeseed meal and GM Soybean MON87701 in Mixed Seed Powder

EURL GMFF Proficiency Testing Report GMFF-21/01

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Executive summary

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) organised a proficiency testing (PT) round (GMFF-21/01) for the determination of GMOs in food and feed materials to support Regulation (EU) 2017/625 on official controls [1]. This PT was open to National Reference Laboratories (NRLs) and EU official control laboratories (OCLs), and a few additional officially appointed laboratories from outside the EU were accepted as well.

Two proficiency test items were distributed to participants to assess the efficacy of GMO analysis in rapeseed meal (T1) and in mixed seed powder (T2). T1 consisted of locally purchased rapeseed meal spiked with MON88302 oilseed rape powder (MON-883Ø2-9). T2 was composed of ground mixed seed powder spiked with GM soybean event MON87701 (MON-877Ø1-2). The EURL GMFF evaluated the homogeneity and stability of the test items and derived the assigned values from in-house measurements. As T1 did not pass the homogeneity criteria, the participants were informed that no *z* score would be attributed to quantitative measurement results on this test item.

Fifty two NRLs from 24 EU Member States and 12 OCLs participated to the exercise.

The results reported for T1 were evaluated for GM event identification and correct compliance assessment in line with the reported (semi-)quantitative results. The results for T2 were rated using *z* and zeta (ζ) scores in accordance with ISO 13528:2015. No $\log_{10}$ transformation of the reported results was performed [2]. A relative standard deviation for proficiency assessment (σ_{pt}) of 25 % was applied, based on the experience acquired from previous PT rounds.

All participants who have sufficiently tested T1 identified the correct GM oilseed rape event in it. Almost all the reported results (95 %) for the content of the GM soybean event MON87701 in T2 were rated as satisfactory according to the *z* score. This confirms that NRLs and enforcement laboratories are able to determine the correct mass fraction of GMOs in the frame of Regulation (EU) 2017/625.

The vast majority of the participants (90 %) correctly evaluated the compliance status of the test items T1, which was not the case for T2 (only 69 %). Several laboratories did not take the measurement uncertainty into account when concluding on compliance.

List of abbreviations and symbols

bp	Base pairs
Ccf	Cruciferin gene target
ddPCR	Droplet Digital Polymerase Chain Reaction
DG SANTE	Directorate General for Health and Food Safety
EC	European Commission
EU	European Union
EURL	European Union Reference Laboratory
FatA(A)	Acyl-ACP thioesterase gene target (specific for A genome of *B. napus*)
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"
$U(x_{pt})$	expanded uncertainty of the assigned value
x_i	mean value reported by participant "i"
x_{pt}	assigned value
z	z performance score
ζ	zeta performance score

1 Introduction

The European Union Reference Laboratory for GM Food and Feed (EURL GMFF), hosted by the Joint Research Centre of the European Commission, organised a proficiency testing (PT) round for the **determination of the mass fractions of MON88302 oilseed rape in rapeseed meal and MON87701 soybean in mixed seed powder** 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 2020, 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).

2 Scope

The presented PT round aimed to assess the performance of NRLs and OCLs in the determination of the mass fractions of GMOs in food and feed products. The PT was mandatory for the NRL/625, recommended for NRL/120, and open to a number of 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 [3] and (EU) No 619/2011 [5].

This PT, organised in line with ISO/IEC 17043:2010 [6], is identified as "**GMFF-21/01**". The PT round was originally planned for launching in 2020 (as GMFF-20/02), but was, due to the restrictions imposed to combat the COVID-19 pandemic, delayed to 2021.

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 participants in this PT received a unique laboratory code used throughout this report. 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. However, the laboratory codes of NRLs appointed in line with Regulation (EU) 2017/625 [1] will be disclosed to DG SANTE 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-21/01 exercise was announced by invitation letters to NRLs and some accepted OCLs on January 6, 2021 (Annex 1). The registration deadline was set to January 22, 2021. Samples were sent to participants on February 9, 2021. Due to the COVID-19 pandemics situation and the temporary closure of a number of laboratories of participants, the reporting deadline was postponed to April 4, 2021.

3.4 Distribution

Each participant received:

- One bottle of test item T1, containing approx. 5 g of powder;
- One bottle of test item T2, containing approx. 5 g of powder;
- One general "Test item accompanying letter.

Samples were dispatched under room temperature conditions.

3.5 Instructions to participants

Detailed instructions were given to participants in the "Instructions letter" (Annex 2).

The test items were described as "*two ground test materials (5 g each), derived from commercially available materials that are not declared as containing GM material*". The testing laboratories were requested to screen the presence of GMOs and assess the compliance of the samples with the applicable GMO legislation.

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:

T1: Rapeseed meal:	Identify <u>any GM rapeseed</u> event(s) in this sample (Semi-)quantify the GM event(s) and assess compliance
T2: Mixed seed powder	Quantify the <u>MON87701 soybean</u> event and assess compliance

Participants were requested to report their calculated mean (x_i) and the associated expanded measurement uncertainty ($U(x_i)$) together with the coverage factor (k) and the analytical technique used for analysis.

Quantitative results had to be reported in mass/mass %. Since the homogeneity study was performed with 200 mg sample intakes, the recommended minimum sample intake was set to 200 mg.

Participants were informed that the procedure used for the analysis should resemble as closely as possible their routine procedures for these types of matrices.

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

Participants were asked to fill in an online questionnaire through EU Survey, accessible with a provided password. The questionnaire was designed to collect additional information related to the measurements and the laboratories, including the detection (qualitative analysis) of the GM events that were requested to be tested.

4 Test item

4.1 Preparation

<u>Test item T1</u> consisted of ground rapeseed meal, purchased from a local rapeseed oil producer in Belgium, later spiked with GM oilseed rape event MON88302. 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). The presence of different crop species and GM events in the rapeseed meal was assessed by using pre-spotted plates for screening [7] and GM event-specific oilseed rape, maize and soybean detection [8]. A contamination of maize and soybean was detected in the rapeseed meal, including three GM soybean events. The results of the event-specific quantitative analysis by dPCR revealed that soybean is present in the rapeseed meal sample at a low level (approximately 0.15 m/m % soybean compared to rapeseed) and that this soybean includes a large fraction (>40 %) of GM soybean, possibly from a soybean stack (e.g. MON87701 x MON89788, approved in the EU) and GTS 40-3-2 soybean (the triple stack does not exist commercially).

The rapeseed meal powder was mixed with MON88302 oilseed rape powder (AOCS CRM 1011-A) in two steps (Table 1) and filled in 5 g portions into 20 ml vials, closed under argon. The final powder had an average particle diameter of 133.6 ± 25.6 µm (k=2, n=3) with a water content of 3.3 ± 0.1 g/100 g (k=2, n=3).

Each vial was identified with the PT identifier and a unique vial number.

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

Characteristic	Rapeseed meal	MON88302 oilseed rape
Type of base material	Crude powder	Powder
Origin	Alvenat, BE	AOCS 1011-A
Grinding equipment	Cryo-grinding vibrating mill	/
Mixing equipment	Turbula mixer, 1h (STEP 1); Dyna-Mix 200, 1h (STEP 2)	
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	7.53 ± 0.21	/
Particle diameter in µm, mean ± *U*[1] (*k*=2, *n*=3)	138.9 ± 8.9	92.5 ± 11.1
Mass used to prepare T1 (g) – STEP 1	91.20	9.00
Mass used to prepare T1 (g) – STEP 2	1400.76	99.45 g of STEP 1

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

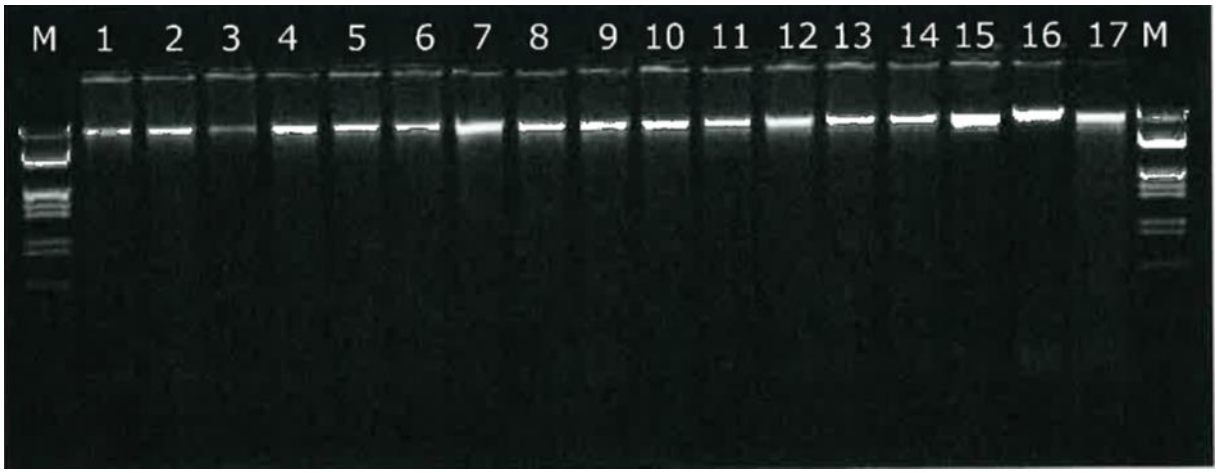

Figure 1. Agarose gel electrophoresis of genomic DNA extracted from the T1 material (lanes 1-14), CRM AOCS 1011-A2 for MON88302 (lane 16), and two CRMs for unrelated projects (lanes 15 and 17). The molecular marker (M) is Lambda DNA DNA/*Eco*RI + *Hind*III (largest band is 21.2 kb).

The amount and the quality of the DNA extracted from the T1 material were verified by UV spectrometry, fluorometry and gel electrophoresis (Figure 1). A CTAB/tip20 method with a sample intake of 200 mg was chosen for homogeneity and stability analyses. The extracted DNA was tested for PCR inhibition on a range of dilutions with both the FatA(A) or Ccf and the MON88302 qPCR assays. Based on the amount of DNA extracted, the DNA fragmentation state (Figure 1) and the absence of PCR inhibition (with a few borderline deviations for the GM event), it was concluded that all DNA extracts contained a sufficient amount of DNA of PCR-grade quality.

Test item T2 was identical to T2 used in EURL-GMFF-PT-02/18. It had been prepared by mixing ground maize and soybean in equal amounts and spiking with Bt11 maize and MON87701 soybean.

Details on the processing and characterisation of this test item can be retrieved from the PT report for EURL-GMFF-PT-02/18 (see report JRC115380 for details, https://gmo-crl.jrc.ec.europa.eu/Proficiency-tests.html). In the current PT round, participants were requested to analyse only the mass fraction of MON87701 in test item T2.

4.2 Homogeneity and stability

Measurements for the homogeneity and stability studies and the statistical treatment of the data were performed by the JRC for T1 and T2, using the corresponding validated event-specific detection methods.

The assessment of homogeneity was performed after the processing and bottling of the test items and before distribution to the participants. Seven bottles were randomly selected and analysed by qPCR in 5 replicates each. Results were evaluated according to ISO 13528:2015 [9]. The contribution from homogeneity (u_{hom}) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated using the software SoftCRM v2.0.21 [10].

The homogeneity measurements were only done for T1, as homogeneity of T2 had already been shown during the preparation of EURL-GMFF-PT-02/18.

The T1 material proved to be insufficiently homogeneous for the GM event (Annex 3.1). This conclusion was triggered by the presence of a much higher GM content measured in an extraction replicate from two bottles. Similarly, deviating results were observed during the stability and characterisation studies. Hence, none of these values were considered as outliers. It was therefore decided not to assess the performance of the participants for the quantification of GM events in the test item T1. Nevertheless, the test item was used to assess the ability of the participants in the identification of the GM event in a rapeseed meal sample and to assess the compliance statement performed by the participants on the basis of their (semi)quantitative measurement results.

To assess the stability of the T1 test item during dispatch conditions (this had already been done for T2 before, see the EURL-GMFF-PT-02/18 report), an isochronous short-term stability study [11] involving two test samples with three replicates each (N=2, n=3) was conducted over one week at +4 °C, +20 °C and +40 °C (3 and 7 days incubation). The measurements by qPCR were performed under repeatability conditions. The results revealed no significant influence of storage at +4 °C, +20 °C or +40 °C on the stability of the test items (compared to storage at -18 °C). The test items were therefore dispatched at ambient temperature.

Before the start of the PT round, the stability of the T2 material, re-used from a previous PT round and stored at 4 °C since 2018, was re-assessed on 2 bottles (in 3 extraction replicates). The slope of the regression line was tested for statistical significance. No significant trend (against similar bottles tested in 2018) was detected at a 99 % confidence level.

The stability of the test items during the extended period covered by the PT round was tested by qPCR for T2 only, analysing the GM content in bottles (N=2, n=3) stored at the normal (+4 °C) storage temperature. 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 99 % confidence level (Annex 3.2). This stability study confirmed that the test item was adequately stable at +4 °C during the whole time period of the PT round (and actually since the preparation of the test item in 2018). The uncertainty contribution to the assigned value due to instability was set to zero (u_{stab}=0) for the investigated analyte [9].

5 Assigned values and corresponding uncertainties

5.1 Assigned values

As mentioned before, no performance score is given for the measurements on T1, due to insufficient homogeneity. An indicative MON88302 content of 0.28 m/m % was measured by the EURL GMFF (Table 3), while a robust mean of the reported results from participants of 0.36 m/m % was calculated.

The assigned value (x_{pt}) for the mass fraction of the MON87701 event in T2 was derived from results reported by the JRC expert laboratories in Geel and Ispra, applying the EURL-validated method. These measurements were performed in 2018 (EURL-GMFF-PT-02/18). Since T2 was proven to be stable, the same assigned value was used in this PT round (Table 3).

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

Sample	GM event	x_{pt}	$u(x_{pt})$	σ_{pt}	$u(x_{pt})/\sigma_{pt}$
Rapeseed meal (T1)	MON88302 oilseed rape	(*0.28*)			
Mixed seed powder (T2)	MON87701 soybean	0.922	0.065	0.231	0.28

5.2 Associated measurement uncertainties

The associated standard measurement uncertainty of the assigned value ($u(x_{pt})$) was calculated following the law of uncertainty propagation, combining the standard measurement uncertainty of the characterisation (u_{char}) with the standard uncertainty contributions from homogeneity (u_{hom}) and stability (u_{stab}), in compliance with ISO 13528:2015 [9]:

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

The uncertainty u_{char} is estimated according to the recommendations of ISO 13528:2015 [9]:

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

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.

Since $u(x_{pt}) < 0.3\sigma_{pt}$ for MON87701 in T2 (Table 3), the standard uncertainty of the assigned value is deemed negligible and need not to be included in the interpretation of the results [9].

5.3 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviation for PT assessment (σ_{pt}) was set to 25 % of the respective assigned value, based on expert judgment (Table 3).

6 Evaluation of results

6.1 Scores and evaluation criteria

Laboratory performance for the (qualitative) identification of the GM event in a test item was scored as follows: D=detected, ND=not detected, NT=test item or GM event not tested. 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 the GM event present in the test items.

The individual laboratory performance for the determination of the GM content was expressed in terms of *z* and ζ scores according to ISO 13528:2015 [9]:

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

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

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

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

x_{pt} is the assigned value;

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

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

The interpretation of the *z* and ζ performance scores is done according to ISO 13528:2015 [9]:

|score| ≤ 2	satisfactory performance	(green in Annex 4)
2 < |score| < 3	questionable performance	(yellow in Annex 4)
|score| ≥ 3	unsatisfactory performance	(red in Annex 4)

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

The **ζ scores** state whether the laboratory's result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value $u(x_{pt})$ and the measurement uncertainty as stated by the laboratory $u(x_i)$. The ζ score includes all parts of a measurement result, namely the expected value (assigned value), its measurement uncertainty in the unit of the result as well as the uncertainty of the reported values. An unsatisfactory ζ score can either be caused by an inappropriate estimation of the concentration, or of its measurement uncertainty, or both.

The standard measurement uncertainty of the laboratory $u(x_i)$ was obtained by dividing the reported expanded measurement uncertainty by the reported coverage factor, *k*. All laboratories in this PT round reported their results with the associated uncertainty and coverage factor.

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

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 reported data from participants were not $\log_{10}$-transformed prior to the performance assessment [2].

6.2 General observations

Overall, 52 NRLs from 24 EU Member States (excluding Estonia, Malta and Ireland; the latter has an agreement with Wageningen Food Safety Research in The Netherlands for GMO analysis) and 12 OCLs registered to this PT round (Table 4). Sixty-two participants reported qualitative and/or quantitative results for T1 and/or T2. Laboratory L58 (OCL) applied only qualitative screening tests, while L03 (OCL) did not provide any results, nor filled in the questionnaire.

The majority of participants applied real-time PCR, while the number of results obtained by digital PCR increased to 9, two times more compared to the previous PT round. More experimental details are listed in Annex 5.

6.3 Laboratory results and scorings

6.3.1 Laboratory performance for GM event identification

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. A number of OCLs are accredited for GMO screening tests only. When positive samples are found under routine operation they are transferred to an NRL for further analysis. These OCLs may have reported one or more quantitative results in this PT round, or they may have decided to provide presence/absence of the GM events only.

The MON88302 GM event in **T1**, like GT73, should react positive in the following screening methods: pFMV, tE9 and CTP2-CP4-EPSPS, while p35S, Tnos, bar or pat elements are absent.

For **T2**, an event identification was not requested as the GM event was mentioned in the instructions. Two OCLs did not report data.

The results are summarised in Table 5, while the experimental details are presented in Annex 4.

All participants having tested the GM event correctly identified it in T1 (Table 5). However, 12 participants did either not assess the T1 test item at all or did not apply the method for the MON83302 event to the T1 material.

Table 4. Overview of participants to GMFF-21/01 by country and category

Country	Participants	NRL/625	NRL/120	OCL (not NRL)
Austria	2	2		
Belgium	3	3		
Bulgaria	3	2		1
Croatia	2	2		
Cyprus	1	1		
Czech Republic	1	1		
Denmark	1	1		
Estonia	0	0		
Finland	2	1	1	
France	2	2		
Germany	18	1	14	3
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	5	4		1
Portugal	1	1		
Romania	1	1		
Serbia	1			1
Slovakia	2	2		
Slovenia	1	1		
Spain	5	2		3
Sweden	1	1		
Turkey	2			2
Total	**64**	**35**	**17**	**12**

Table 5. Qualitative identification of the GM event present in T1

Event tested?	Outcome	MON88302
Tested	Detected (D)	52
	Not detected (ND)	0
Not tested (NT)		12
Total		64

6.3.2 Laboratory performance for quantification

Laboratory performance for quantification of the GM event in test item T2 (only) was expressed in terms of *z* and ζ scores. Satisfactory performance scores are highlighted in green, questionable in yellow, unsatisfactory in red (Figure 2, Annex 4). Cells were left uncoloured when the outcome could not be evaluated (Annex 4).

Annex 4 presents the reported results as table and graph for each measurand. The corresponding Kernel density plot (T2 only) has been obtained by using the software available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [12].

Figure 2 summarises the performance scores obtained. A total of 62 quantitative results were reported for MON87701 in T2 and have been scored.

An overall satisfactory performance of 95 % (59 out of 62 reported results), expressed as *z* score, was obtained for the quantification of MON87701 in T2. Only 3 questionable and no unsatisfactory results were found.

When taking into account the reported measurement uncertainties, 8 unsatisfactory scores (expressed as ζ score) were obtained. Six of them were reported by OCLs, and 2 by NRL/625. Six participants reported a significantly underestimated result associated with a low measurement uncertainty, while the two others reported an overestimated value without any measurement uncertainty.

Despite the fact that participants were only requested to "semi-quantify" the MON88302 event in T1 (proven to be insufficiently homogeneous), most of them reported results close to the indicative value (Table 3), which was further confirmed by the close agreement between the indicative value and the consensus robust mean.

MON87701 soybean

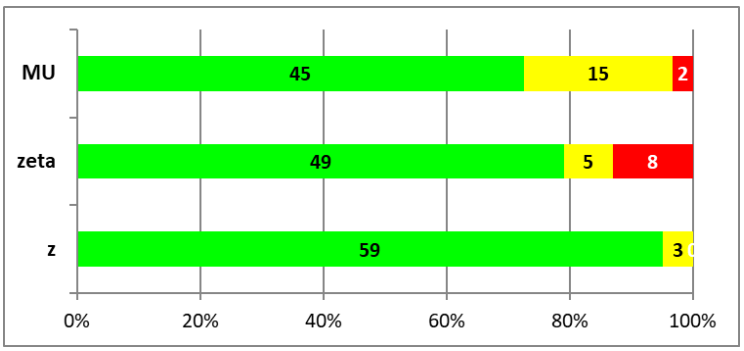

Figure 2.
Overview of laboratory performance according to *z* and ζ scores, for the content of the GM event in test item T2. Corresponding numbers of laboratories are shown in the bars. Satisfactory, questionable and unsatisfactory performance scores are indicated in green, yellow and red, respectively. Measurement uncertainty was evaluated as follows:
Case "a" (green): $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$
Case "b" (yellow): $u_{rel}(x_i) < u_{rel}(x_{pt})$
Case "c" (red): $u_{rel}(x_i) > \sigma_{pt,\%}$

6.3.3 Truncated values

No truncated values were reported for T2, while four truncated values were reported for T1, which were not assessed against the indicative value.

6.3.4 Measurement uncertainties

All laboratories having reported quantitative results, except 2 OCLs, provided expanded measurement uncertainties and coverage factors for T2. One NRL/625 provided an uncertainty without a coverage factor.

The measurement uncertainties were evaluated according to ISO 13529 [9] (See section 6.1). Most of the laboratories (73 %) reported a realistic measurement uncertainty (Case "a" in Figure 2). Laboratory L64 (NRL/625) reported a relative measurement uncertainty (in % of the reported value), and acknowledged his mistake by email to the PT organiser.

6.3.5 Compliance statement

Regulation (EC) No 1829/2003 [3] 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 [5] 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.

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 all GM events present in T1 or T2 are authorised in the EU, the reported range (result ± expanded uncertainty) is to be compared to the labelling threshold of 0.9 m/m%.

A total of 60 and 53 participants filled in the questions regarding compliance of T1 and T2, respectively. Most of them also provided a justification for their choice among the 5 compliance options (see above). The option selected and the justification provided were evaluated.

An example of a correct compliance assessment is provided below for sample T1 and T2.

Test item T1

The following assumptions were taken into account:

- The GM event in T1 is an authorised GM event in the EU, hence the labelling threshold to be applied is 0.9 m/m % [3].
- The content of MON88302 measured in T1 is expected to be below the labelling threshold, based on the indicative value provided and taking into consideration the measurement uncertainty ($x - U \leq$ Threshold).
- This material is to be considered as "Compliant because no labeling required" (CNL) to Regulation (EC) No 1829/2003 (CNL).

Table 6 summarises the statements reported for T1, taking into account the reported analytical results (or lack of results). The majority of the laboratories (45 out of 52 responses, i.e. 87 %) correctly interpreted the compliance rules based on their obtained measurement results. Only one participant (L62, OCL) selected the wrong justification for the non-compliance statement (as the reported result was < 0.9 %). Two other NRLs reported that the sample was also compliant under Regulation 619/2011, whereas this Regulation does not apply to GMOs that are authorised in the EU. One OCL (L63) concluded, additionally to the correct CNL statement, on non-compliance under Regulation 619/2011, which is wrong for the same reason. Two participants selected that the samples should have been labeled because of the identification of other GM events besides MON88302, which were found to be present at a content > 0.9 m/m %. This is in line with our own finding of the presence of traces of three GM soybean events during processing of the rapeseed meal, as reported in Section 4.1. Overall, 90 % of the compliance statements (i.e. 47 out of 52) were considered as correct.

Table 6. Reported compliance statements for T1 (rapeseed meal)

Compliance Statement	Laboratory Measurement	Number of Laboratories	Comment
Compliant, because no labelling required	x ± U ≤ 0.9 m/m %	45	Correct based on the result
Compliant, under Regulation 619/2011 but <0.1 m/m %	x ± U ≤ 0.1 m/m %	2	Wrong because Regulation does not apply here
Not compliant, should have been labelled	x ± U > 0.9 m/m %	0	
	x ± U ≤ 0.9 m/m %	2	Based on presence of other GM events
Not compliant, under Regulation 619/2011 and >0.1 m/m %	x ± U ≤ 0.9 m/m %	1	Wrong because Regulation does not apply here
Cannot be concluded / not quantified		5	
Total no. of participants that provided a statement		52*	

* Some participants reported compliance statements for both Regulations 1829/2003 and 619/2011

Test item T2

A similar evaluation of the reported compliance statements was done for T2, containing MON87701 soybean, which was labelled as feed:

- The MO87701 event is an authorised GM event in the EU, hence the labelling threshold to be applied is 0.9 m/m % [3].
- Knowing that the assigned (expanded) range is 0.92 ± 0.13 (k=2) m/m %, and since 0.92 – 0.13 = 0.79 m/m %, which is below the labelling threshold (x - U ≤ Threshold)
- This material is to be considered "Compliant because no labelling required" (CNL).

Many participants (35 out of 59, *i.e.* 59 %) correctly considered the sample as compliant (Table 7) and selected the correct reason for compliance, *i.e.* that the event is authorised under Regulation (EC) No 1829/2003 and its content was below 0.9 m/m %. Another 18 participants concluded that the sample should have been labeled, whereas this option was correct for only 6 participants (x_i- U > 0.9 m/m %). Many of the other participants that selected this option did not consider that the expanded measurement uncertainty reported for their result has to be considered. It therefore cannot be unambiguously concluded that the GMO is present at a level above the labeling threshold. Four participants referred also to Regulation 619/2011, which is not applicable for this GM event. One NRL (L32) concluded that compliance could not be decided as it was not known if the MON87701 presence was adventitious or technically unavoidable. This is considered correct, although it was the intention of this exercise to evaluate compliance on the basis of the obtained results only. Six participants could not conclude on compliance, although their results clearly showed that the GM event was present below 0.9 m/m %. Overall, 69 % of the compliance statements (i.e. 41 out of 59) were considered as correct.

Table 7. Reported compliance statements for T2 (mixed seed powder)

Compliance Statement	Laboratory Measurement	Number of Laboratories	Comment
Compliant, because no labelling required	x ± U ≤ 0.9 m/m %	35	Correct based on the result
Compliant, under Regulation 619/2011 but <0.1 m/m %	x ± U ≤ 0.1 m/m %	3	Wrong because Regulation does not apply here
Not compliant, should have been labelled	x ± U > 0.9 m/m %	6	Correct based on the result
	x ± U ≤ 0.9 m/m %	12	Wrong, as U not considered
Not compliant, under Regulation 619/2011 and >0.1 m/m %	x ± U ≤ 0.9 m/m %	1	Wrong because Regulation does not apply here
Cannot be concluded / not quantified		6	
Total no. of participants that provided a statement		59*	

* Some participants reported compliance statements for both Regulations 1829/2003 and 619/2011

6.3.6 Additional information extracted from the questionnaire

The questionnaire was answered by 60 participants. Annex 5 summarises the experimental details provided by each participant.

The majority of participants (67 %) reported that their laboratory was accredited in accordance with ISO/IEC 17025 for the methods used in the PT round, and 25 % of respondents have only accreditation for some of the methods used. Five laboratories reported that the methods used for reporting their results are not within their scope of accreditation under ISO/IEC 17025:2017. For both test items, approximately 40 % of the participants applied a DNA extraction method involving lysis with (1 % or 2 %) CTAB, while over 50 % used one of several commercial kits for DNA extraction, mostly NucleoSpin Food. The sample intake for extraction was about 200-300 mg for most laboratories, but 12 laboratories used 500 mg or more and 7 laboratories used less than 200 mg for one or both test items, contrary to the recommended minimum sample intake. Most laboratories used screening methods to limit the number of GMOs to test by event-specific methods. The most common screening strategy, used by over 40 laboratories, involved testing for P35S and Tnos, often in combination with PAT and *bar*. Given that there are only 6 rapeseed GM events in the GM register (authorised or with pending authorisation in the EU), a number of participants directly used the event-specific methods for these events, without first applying screening tests.

Interestingly, 9 laboratories, all from Germany, applied dPCR for the quantification of the MON87701 content in T2, and one laboratory from Belgium used both qPCR and dPCR. Most of these participants, except one, obtained a satisfactory *z* score, indicating the potential of this technique for GMO quantification.

7 Conclusions

The proficiency test GMFF-21/01 was organised to assess the analytical capabilities of EU NRLs and OCLs to identify event MON88302 in rapeseed meal (T1) and to determine the content of MON87701 in a mixed seed powder (T2).

All participants who tested for the presence of MON88302 in T1 identified the event (100 % satisfaction rate). While the quantitative results reported for this event were not scored, most of the results obtained were consistent around the provided indicative value.

The overall performance of the participants for the determination of the GM event in T2 was very good, *i.e.* 95 % satisfactory *z* scores were obtained for the content of MON87701 soybean in a maize/soybean seed mixture. Compared to the previous PT round that used the same test item (EURL-GMFF-PT-02/18), this is an increase by 8 % (from 87 %). It confirms the improved analytical capabilities to enforce Regulation (EU) 2017/625 that is observed over the past 10 years of proficiency testing by the EURL GMFF [14]. Also the estimation of the measurement uncertainty has improved for the same GM event from 55 % in 2018 to 72 % (realistic measurement uncertainty, code “a” in Annex 4) in the current PT round.

A total of 90 % and 69 % of the participants correctly evaluated the compliance status of the test items T1 and T2, respectively. However, a number of participants, including several NRLs mandated with a control function (NRL/625), did not properly take the measurement uncertainty into account when concluding about regulatory compliance of a sample.

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
BioGEVES	FRANCE
Service Commun des Laboratoires	FRANCE
Bavarian Health and Food Safety Authority (LGL)	GERMANY
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)	GERMANY
CVUA Freiburg	GERMANY
Federal Office for Consumer Protection and Food Safety	GERMANY
German Federal Institute for Risk Assessment	GERMANY
Institute for Hygiene and Environment	GERMANY
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF) M-V	GERMANY
Landesamt für Umweltschutz Sachsen-Anhalt	GERMANY
Landesamt für Verbraucherschutz Sachsen-Anhalt	GERMANY
Landeslabor Berlin-Brandenburg	GERMANY
Landeslabor Schleswig-Holstein	GERMANY
Landesuntersuchungsanstalt fuer das Gesundheits- und Veterinärwesen Sachsen	GERMANY
LAVES-LVI Braunschweig/Hannover	GERMANY
LTZ Augustenberg	GERMANY
LUFA Speyer	GERMANY
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GERMANY
Thueringer Landesamt fuer Verbraucherschutz	GERMANY
Thüringer Landesamt für Landwirtschaft und Ländlichen Raum	GERMANY
General Chemical State Laboratory	GREECE
Biomi Kft	HUNGARY
National Food Chain Safety Office	HUNGARY
CREA Centro di Ricerca Difesa e Certificazione	ITALY
Italian Institute of Health (ISS)	ITALY
Istituto Zooprofilattico Sperimentale Lazio e Toscana	ITALY
Institute of Food Safety, Animal Health and Environment „BIOR"	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
Laboratoire National de Santé	LUXEMBOURG
Wageningen Food Safety Research (WFSR)	NETHERLANDS
Instytut Zootechniki PiB KLP Pracownia w Szczecinie	POLAND
National Veterinary Research Institute	POLAND
Plant Breeding and Acclimatization Institute NRI	POLAND
Regional Laboratory of Genetically Modified Food	POLAND
Wojewódzki Inspektorat Weterynarii w Opolu	POLAND
INIAV, I.P.	PORTUGAL
Institute of Diagnosis and Animal Health	ROMANIA

Organisation	Country
SP Laboratorija a.d.	SERBIA
Central Control and Testing Institute of Agriculture, Bratislava	SLOVAKIA
State Veterinary and Food Institute, VFI in Dolny Kubin	SLOVAKIA
National Institute of Biology	SLOVENIA
Centro Nacional de Alimentación. AESAN	SPAIN
Gobierno de Navarra	SPAIN
Instituto de Ciencias de la Salud Consejeria de Sanidad Junta de Munidades de Castilla La Mancha	SPAIN
Laboratorio Arbitral Agroalimentario - MAPA	SPAIN
Laboratorio Central de Veterinaria	SPAIN
Livsmedelsverket (National Food Agency)	SWEDEN
Ankara Food Control Laboratory	TURKEY
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

EURL European Union Reference Laboratory for GM Food & Feed

Geel, 6 January 2021
JRC.F.5/HE/wb/ARES(2021) 21-001

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-21/01

Dear Colleague,

Hereby, I would like to invite you for participating to the proficiency test (PT) GMFF-21/01, 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. I would like to remind you that participation is mandatory for all NRLs designated under Regulation (EU) 2017/625 and recommended for NRLs nominated under Regulation (EU) No 120/2014. Please note that this invitation is only sent to the NRLs. You are allowed to distribute this letter to any official laboratory within your network of control laboratories for which you deem its participation as useful; they may register for this PT using the registration details provided in this letter.

This PT will include two ground test materials (5 g each), processed by the JRC and "*derived from products that are not declared as containing GM material*". The testing laboratories are requested to confirm the absence of GMOs and assess the compliance of the samples with the applicable GMO legislation. The following tasks are requested from the participants:

Test Item 1: Rapeseed meal
- Identify the GM rapeseed event(s) in this sample
- (Semi-)quantify the GM event(s) and assess compliance.

Test Item 2: Mixed seed powder
- Quantify the MON87701 soybean event and assess compliance.

Note that performance scores (z and ζ scores) will be determined for T2 only, not for T1, because the homogeneity of T1 is not fully guaranteed at the usual sample intake level of 200 mg. However, you are asked to assess the compliance of the sample based on your qualitative and (semi-)quantitative analysis results for this test item (which have to be reported in order to evaluate your compliance statement).

Participants are reminded to use their routine approaches for GMO testing, taking care to ensure that the DNA extraction procedure used is adapted to the sample matrix and that the quality of the DNA obtained is suitable for PCR. The quantitative results have to be reported in mass/mass %. Further details on your analysis have to be reported in a questionnaire via an online EU Survey.

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 (please enter your details in small letters with first letter capitalised):

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=2621

Following registration, you are requested to return the signed registration form to us by e-mail, as scanned pdf (not by fax, please). Please be aware that each laboratory can only register once for this PT.

The deadline for registration is **Friday 22 January 2021**.

The test items will be shipped on **9 February 2021**. You are requested to inform us promptly if you have not received the samples by 19 February 2021.

The deadline for submission of the results is (at least) 6 weeks after dispatch. The exact date will be communicated in the letter accompanying the test items.

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

The EURL GMFF is looking forward to your participation!

Yours sincerely,

e-signed

Prof. Dr. Hendrik Emons
Head of Unit

Cc: Wim Broothaerts, PT coordinator

Contact:

European Reference Laboratory for GM Food and Feed
Dr Wim Broothaerts, Project leader GMO Control
European Commission
Joint Research Centre – Retieseweg 111, B-2440 Geel, Belgium
Telephone: direct line (+32) 14 57 1612
JRC-EURL-GMFF-CT@ec.europa.eu

Annex 2: Instructions letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

European Union Reference Laboratory for GM Food & Feed

Geel, 8 February 2021
JRC.F.5/WB/mt ARES(2020)

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

Subject: **Instructions for GMFF-21/01, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* rapeseed meal and mixed seed powder**

Dear Dr «Surname»,

Thank you for participating to GMFF-21/01. In one of the following days you should receive two test materials, T1 and T2, containing approximately 5 g of ground sample. These should be store in a dark and cold place (at approximately 4 ºC) until use.

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 (except for T1 – see below).

The two ground test materials are "*derived from imported samples that are not declared as containing GM material*". The testing laboratories are requested to confirm the absence of GMOs and assess the compliance of the samples with the applicable GMO legislation. The following tasks are requested from the participants:

Tasks
Test Item 1: Rapeseed meal
- Identify any GM rapeseed event(s) in this sample
- (Semi-)quantify the GM event(s) and assess compliance.

Test Item 2: Mixed seed powder
- Quantify the MON87701 soybean event and assess compliance.

Note that performance scores (z and ζ scores) will be determined for T2 only, not for T1, because the homogeneity of T1 is not fully guaranteed at the usual sample intake level of 200 mg. However, you are asked to assess the compliance of the sample based on your qualitative (presence/absence) and (semi-) quantitative analysis results for this test item (results have to be reported in order to evaluate your compliance statement).

To correctly assess the performance of your laboratory, the procedures used for detection/quantification of the GM events should resemble as closely as possible the ones used in routine sample analysis.

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 need to be reported **with two decimal places (e.g. 0.64 or 1.29)**:

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

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 *z* scores, the uncertainty reported will be considered in the evaluation of the results using ζ (zeta) scores (for T2 only).

You can find the MILC reporting website at https://web.jrc.ec.europa.eu/ilcReportingWeb. You need a personal password to access this webpage which is **«Part_key»**. 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/PT21-01, enter the password (**PT2101**), and answer the questions of the survey. This survey includes questions on the analytical approaches used, and a statement on compliance to EU legislation. Submit your answers to the survey on-line (no need to send them by e-mail). For the **compliance declaration** you should assume that the samples were not labelled as containing GMOs and that any GMO presence would be adventitious or technically unavoidable.

The deadline for the submission of the results and the questionnaire is Friday 26 March 2020. It will not be possible to submit your results after the deadline.

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

You should keep the test items at approximately 4 °C in order to voluntary repeat the analysis in case of an unsatisfactory performance. Please, dispose the test items thereafter.

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

Thank you for the collaboration in this PT.

Yours sincerely,

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

Annex 3: Homogeneity and stability results

3.1 Homogeneity

Homogeneity of MON88302 oilseed rape in T1

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
1	0.33	0.23	0.83	0.46	0.23
18	0.25	0.22	0.23	0.42	0.29
35	0.3	0.17	0.16	0.26	0.17
69	0.18	0.59	0.24	0.24	0.31
73	0.28	0.34	0.33	0.29	0.24
98	0.2	0.2	0.2	0.18	0.26
112	0.17	0.31	0.33	0.23	0.18
Mean	0.28				
s_x	0.07				
s_w	0.12				
S_s	0.05				
u^*	0.03				
σ_{pt}	0.07				
$0.3 * \sigma_{pt}$	0.02				
$s_s \leq 0.3 * \sigma_{pt}$	NO				
Assessment	NOT Passed				

Homogeneity of MON87701 soybean in T2 (assessed as part of EURL-GMFF-PT-02/18)

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
17	1	0.95	0.93	1.22	0.93
41	1.07	1.2	0.96	1.31	1.04
80	0.94	0.95	1.01	0.89	1
142	0.99	1.02	0.88	0.85	0.88
185	1	1.08	0.97	0.91	0.9
197	0.8	1.18	0.84	0.92	0.9
250	1.06	0.84	0.86	0.84	1.12
Mean	0.98				
s_x	0.07				
s_w	0.11				
S_s	0.04				
u^*	0.03				
σ_{pt}	0.23				
$0.3 * \sigma_{pt}$	0.07				
$S_s \leq 0.3 * \sigma_{pt}$	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 [13].

All values are in m/m %

3.2 Stability

In the table below, the stability was assessed according to ISO 13528:2015 § B.5 [9].

Stability MON87701 soybean in T2 (qPCR) (all values are in m/m %)

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	11	0.84	0.89	0.96	0.97
	158	0.86	1.19	1.10	
130	28	0.86	0.86	0.89	0.90
	240	0.90	0.98	0.89	

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

Stability: passed

Annex 4: Results and laboratory performance

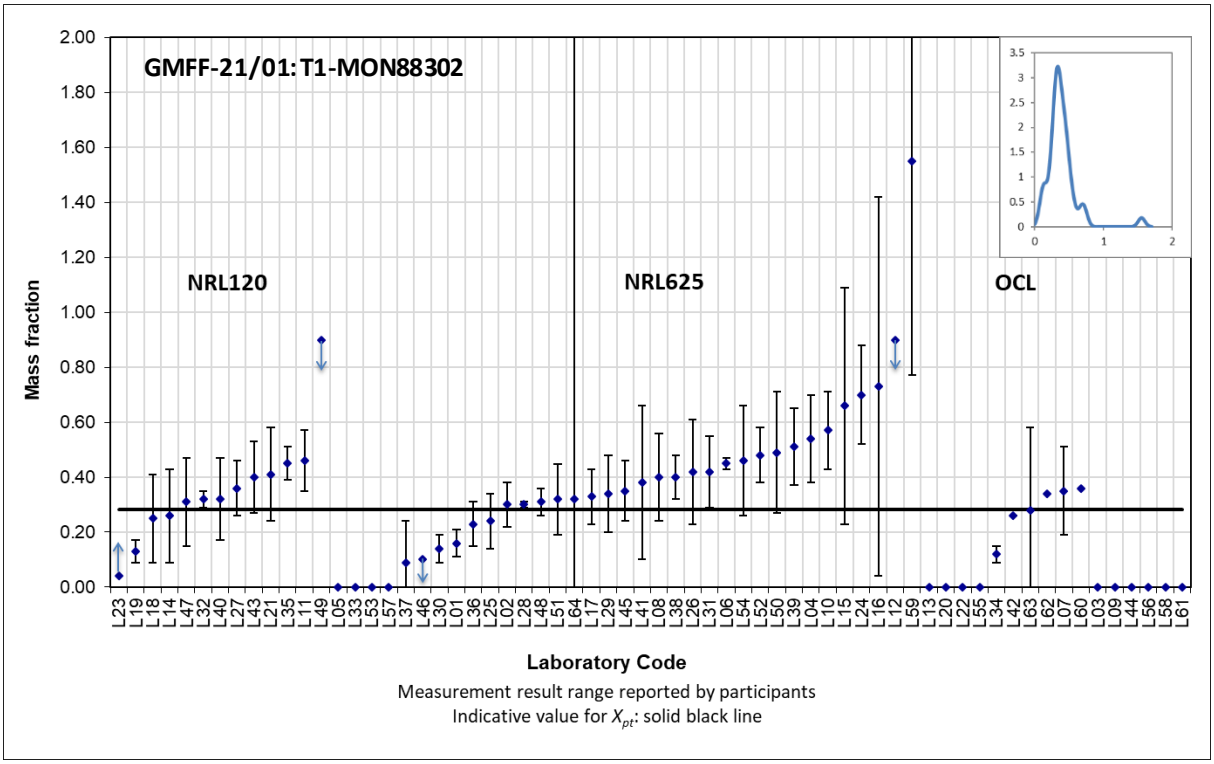
- The PT coordinator set the measurement uncertainty $u(x_i)$ to zero when no expanded uncertainty was reported
- The PT coordinator set k = 1.73 when no coverage factor (k) was reported
- Performance scores (z and ζ - satisfactory, questionable, unsatisfactory
- Measurement uncertainty (MU): a: $u(x_{pt,rel}) \leq u(x_i) \leq \sigma_{pt}$; b: $u(x_i) < u(x_{pt})$; c: $u(x_i) > \sigma_{pt}$
- ID = GM event identification (D = detected, ND = not detected, NT = not tested).
- Compl. = Compliance statement (shown in **bold red** if considered wrong):
  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.

MON88302 oilseed rape in T1

Lab Code	Type	x_i	±	k	Technique	ID	Compl.
L01	NRL/625	0.16	0.05	2	RT-PCR	D	CNL
L02	NRL/625	0.3	0.08	2	RT-PCR	D	CNL
L03	OCL					NT	
L04	NRL/625	0.54	0.16	2	RT-PCR	D	CNL
L05	NRL/120					NT	
L06	NRL/625	0.45	0.02	2	RT-PCR	D	CNL
L07	OCL	0.35	0.16	2	RT-PCR	D	CNL
L08	NRL/625	0.4	0.16	2	RT-PCR	D	CNL
L09	OCL					NT	**CNL**
L10	NRL/625	0.57	0.14	3	RT-PCR	D	CNL
L11	NRL/120	0.46	0.11	2	dPCR	D	CNL
L12	NRL/625	< 0.9			RT-PCR	D	CNL
L13	NRL/625					NT	
L14	NRL/120	0.26	0.17	2.365	RT-PCR	D	CNL
L15	NRL/625	0.66	0.43	2	RT-PCR	D	CNL
L16	NRL/625	0.73	0.69	2	RT-PCR	D	CNL, **C<LLP**[1]
L17	NRL/625	0.33	0.1	2	RT-PCR	D	CNL
L18	NRL/120	0.25	0.16	2	RT-PCR	D	
L19	NRL/120	0.13	0.04	2	RT-PCR	D	CNL
L20	NRL/625					D	CNL
L21	NRL/120	0.41	0.17	2	RT-PCR	D	CNL
L22	NRL/625					NT	
L23	NRL/120	> 0.04			RT-PCR	D	CNC
L24	NRL/625	0.7	0.18	2	RT-PCR	D	CNL
L25	NRL/625	0.24	0.1	2		D	CNL
L26	NRL/625	0.42	0.19	2	RT-PCR	D	CNL
L27	NRL/120	0.36	0.1	2	RT-PCR	D	CNL
L28	NRL/625	0.3	0.01	2	RT-PCR	D	NCL[2]
L29	NRL/625	0.34	0.14	2	RT-PCR	D	CNL
L30	NRL/625	0.14	0.05	1.73	RT-PCR	D	CNL
L31	NRL/625	0.42	0.13	2	RT-PCR	D	CNL
L32	NRL/120	0.32	0.03	2.36	dPCR	D	CNL
L33	NRL/120					NT	CNC
L34	OCL	0.12	0.03	2	dPCR	D	CNL
L35	NRL/120	0.45	0.06	2.57	RT-PCR	D	CNL
L36	NRL/625	0.23	0.08	2	RT-PCR	D	CNL
L37	NRL/625	0.09	0.15	2	RT-PCR	D	CNC[2]
L38	NRL/625	0.4	0.08	2	RT-PCR	D	CNL

Lab Code	Type	x_i	±	k	Technique	ID	Compl.
L39	NRL/625	0.51	0.14	2		D	CNL
L40	NRL/120	0.32	0.15	2	RT-PCR	D	CNL[2]
L41	NRL/625	0.38	0.28	2	RT-PCR	D	CNL
L42	OCL	0.26	0.001	2	RT-PCR	D	CNL
L43	NRL/120	0.4	0.13	2	RT-PCR	D	CNL, **C<LLP[1]**
L44	OCL					NT	
L45	NRL/625	0.35	0.11	2	RT-PCR	D	
L46	NRL/625	< 0.1			RT-PCR	D	CNL
L47	NRL/120	0.31	0.16	2	RT-PCR	D	CNL
L48	NRL/625	0.31	0.05	2		D	CNL
L49	NRL/120	< 0.9			RT-PCR	D	CNL
L50	NRL/625	0.49	0.22	2	RT-PCR	D	CNL
L51	NRL/625	0.32	0.128	2	RT-PCR	D	CNL
L52	NRL/625	0.48	0.1	2	RT-PCR	D	CNL
L53	NRL/120					NT	
L54	NRL/625	0.46	0.2	2	RT-PCR	D	CNL
L55	NRL/625					NT	
L56	OCL					NT	CNC
L57	NRL/120					NT	
L58	OCL					NT	
L59	NRL/625	1.548	0.776	2		D	CNL
L60	OCL	0.36			RT-PCR	D	
L61	OCL					D	CNC
L62	OCL	0.34			RT-PCR	D	**NCL**
L63	OCL	0.28	0.3	2	RT-PCR	D	CNL, **NC>LLP[1]**
L64	NRL/625	0.32	20	2	RT-PCR	D	CNL

[1] Compliance to Regulation 619/2011 should not have been indicated, as this regulation does not apply to authorised GMOs
[2] Laboratory communicated that there are other GM events present in the sample besides MON88302, and therefore the compliance to Regulation 1829/2003 could not be concluded

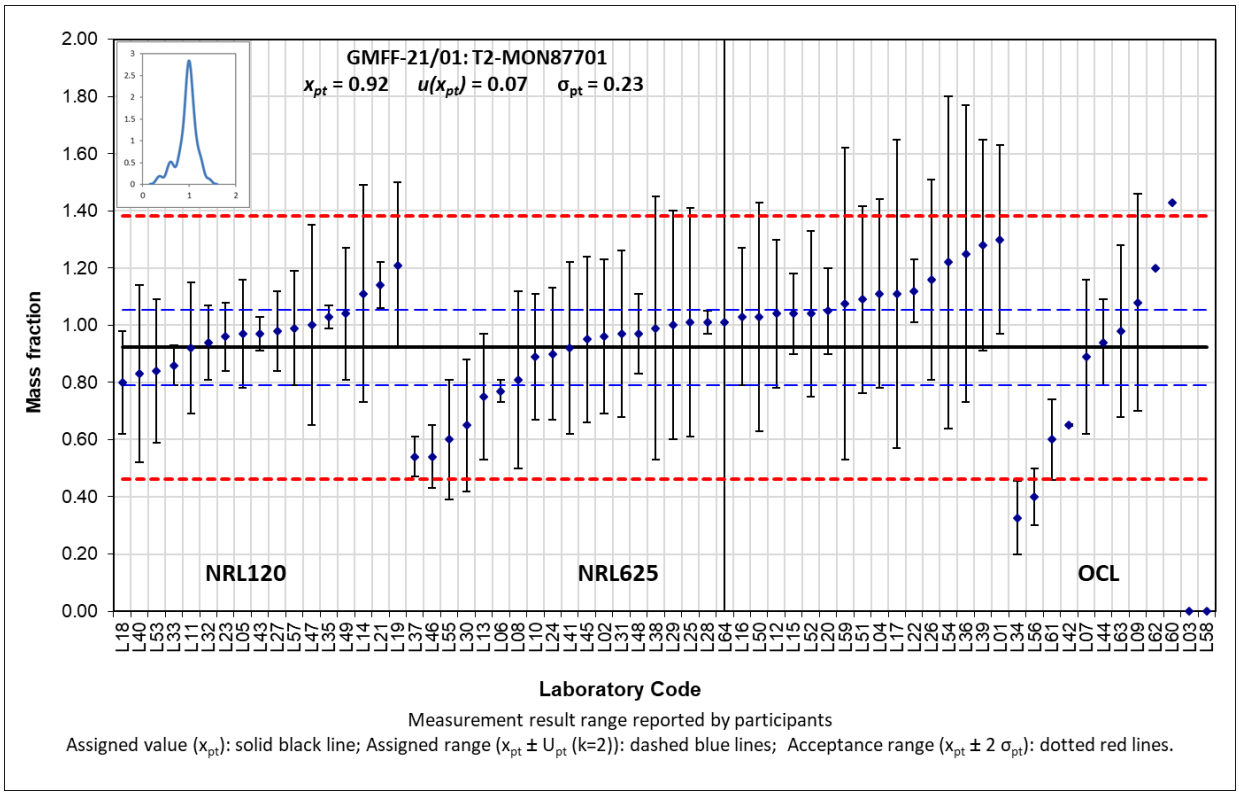


MON87701 soybean in T2

Lab Code	Type	x_i	±	k	Technique	$u(x_i)$	z score	ζ score	MU	Compl.
L01	NRL/625	1.3	0.33	2	RT-PCR	0.165	1.64	2.13	a	NCL
L02	NRL/625	0.96	0.27	2	RT-PCR	0.135	0.16	0.25	a	CNL
L03	OCL									
L04	NRL/625	1.11	0.33	2	RT-PCR	0.165	0.81	1.06	a	NCL
L05	NRL/120	0.97	0.19	2	dPCR	0.095	0.21	0.41	a	CNL
L06	NRL/625	0.77	0.04	2	RT-PCR	0.020	-0.66	-2.23	b	CNL
L07	OCL	0.89	0.27	2	RT-PCR	0.135	-0.14	-0.22	a	CNL
L08	NRL/625	0.81	0.31	2	RT-PCR	0.155	-0.49	-0.67	a	CNC
L09	OCL	1.08	0.38	2	RT-PCR	0.190	0.68	0.78	a	NCL
L10	NRL/625	0.89	0.22	3	RT-PCR	0.073	-0.14	-0.33	a	CNL
L11	NRL/120	0.92	0.23	2	dPCR	0.115	-0.01	-0.02	a	CNL
L12	NRL/625	1.04	0.26	2	RT-PCR	0.130	0.51	0.81	a	CNL
L13	NRL/625	0.75	0.22	2		0.110	-0.75	-1.35	a	CNL
L14	NRL/120	1.11	0.38	2.365	RT-PCR	0.161	0.81	1.08	a	NCL
L15	NRL/625	1.04	0.14	2	dPCR	0.070	0.51	1.23	b	CNL
L16	NRL/625	1.03	0.24	2	RT-PCR	0.120	0.47	0.79	a	CNL, C<LLP[1]
L17	NRL/625	1.11	0.54	2	RT-PCR	0.270	0.81	0.68	a	CNL
L18	NRL/120	0.8	0.18	2	RT-PCR	0.090	-0.53	-1.10	a	
L19	NRL/120	1.21	0.29	2	RT-PCR	0.145	1.25	1.81	a	NCL
L20	NRL/625	1.05	0.15	2		0.075	0.55	1.28	a	NCL
L21	NRL/120	1.14	0.08	2	dPCR	0.040	0.94	2.84	b	NCL
L22	NRL/625	1.12	0.11	2	RT-PCR	0.055	0.86	2.32	b	NCL
L23	NRL/120	0.96	0.12	1.96	RT-PCR	0.061	0.16	0.42	b	CNL
L24	NRL/625	0.9	0.23	2	RT-PCR	0.115	-0.10	-0.17	a	CNL
L25	NRL/625	1.01	0.4	2		0.200	0.38	0.42	a	CNL
L26	NRL/625	1.16	0.35	2	RT-PCR	0.175	1.03	1.27	a	CNL
L27	NRL/120	0.98	0.14	2	dPCR	0.070	0.25	0.60	a	CNC
L28	NRL/625	1.01	0.04	2	RT-PCR	0.020	0.38	1.28	b	NCL
L29	NRL/625	1	0.4	2	RT-PCR	0.200	0.34	0.37	a	CNL
L30	NRL/625	0.65	0.23	1.73	RT-PCR	0.133	-1.18	-1.84	a	CNL
L31	NRL/625	0.97	0.29	2	RT-PCR	0.145	0.21	0.30	a	CNL
L32	NRL/120	0.94	0.13	2.36	dPCR	0.055	0.08	0.21	b	CNC[2]
L33	NRL/120	0.86	0.07	2.13	dPCR	0.033	-0.27	-0.85	b	NCL
L34	OCL	0.327	0.13	2	dPCR	0.065	-2.58	-6.46	a	CNL
L35	NRL/120	1.03	0.04	2.57	RT-PCR	0.016	0.47	1.60	b	CNL
L36	NRL/625	1.25	0.52	2	RT-PCR	0.260	1.42	1.22	a	CNC
L37	NRL/625	0.54	0.07	2	RT-PCR	0.035	-1.66	-5.16	b	CNL
L38	NRL/625	0.99	0.46	2	RT-PCR	0.230	0.29	0.28	a	CNL
L39	NRL/625	1.28	0.37	2		0.185	1.55	1.82	a	NCL
L40	NRL/120	0.83	0.31	2	RT-PCR	0.155	-0.40	-0.55	a	CNC
L41	NRL/625	0.92	0.3	2	RT-PCR	0.150	-0.01	-0.01	a	CNL
L42	OCL	0.65	0.0028	2		0.001	-1.18	-4.17	b	CNL
L43	NRL/120	0.97	0.06	2	RT-PCR	0.030	0.21	0.66	b	NCL, C<LLP[1]
L44	OCL	0.94	0.15	2	RT-PCR	0.075	0.08	0.18	a	NCL
L45	NRL/625	0.95	0.29	2	RT-PCR	0.145	0.12	0.17	a	
L46	NRL/625	0.54	0.11	2	RT-PCR	0.055	-1.66	-4.48	a	CNL
L47	NRL/120	1	0.35	2	RT-PCR	0.175	0.34	0.42	a	CNL
L48	NRL/625	0.97	0.14	2		0.070	0.21	0.50	a	NCL

Lab Code	Type	*xi*	±	*k*	Technique	*u(xi)*	*z* score	ζ score	MU	Compl.
L49	NRL/120	1.04	0.23	3.18	dPCR	0.072	0.51	1.21	b	CNL
L50	NRL/625	1.03	0.4	2	RT-PCR	0.200	0.47	0.51	a	CNL
L51	NRL/625	1.09	0.327	2	RT-PCR	0.164	0.73	0.95	a	NCL
L52	NRL/625	1.04	0.29	2	RT-PCR	0.145	0.51	0.74	a	CNL
L53	NRL/120	0.84	0.25	2	RT-PCR	0.125	-0.36	-0.58	a	CNL
L54	NRL/625	1.22	0.58	2		0.290	1.29	1.00	a	CNL
L55	NRL/625	0.6	0.21	2	RT-PCR	0.105	-1.40	-2.61	a	CNL, C<LLP[1]
L56	OCL	0.4	0.1	2	RT-PCR	0.050	-2.27	-6.35	a	CNC
L57	NRL/120	0.99	0.2	2	RT-PCR	0.100	0.29	0.57	a	CNL
L58	OCL				other					
L59	NRL/625	1.076	0.546	2		0.273	0.67	0.55	c	CNL
L60	OCL	1.43			RT-PCR	0.000	2.20	7.78	b	
L61	OCL	0.6	0.14	2	RT-PCR	0.070	-1.40	-3.37	a	CNL
L62	OCL	1.2			RT-PCR	0.000	1.20	4.25	b	NCL
L63	OCL	0.98	0.3	2	RT-PCR	0.150	0.25	0.35	a	NCL, NC>LLP[1]
L64	NRL/625	1.01	18	2	RT-PCR	9.000	0.38	0.01	c	NCL

[1] Compliance to Regulation 619/2011 should not have been indicated, as this regulation does not apply to authorised GMOs
[2] Laboratory could not decide on compliance of T2 as it was not known if MON87701 presence was adventitious or technically unavoidable



Annex 5: Results of the questionnaire

The answers to the questionnaire are summarised in the table below. The following abbreviations are used for the questions and answers:

Column	Description
Lab	Labcode
T1, T2	T1, T2 tested? Y=yes, N=No
Accr	Are the methods used for reporting your results within the scope of accreditation under ISO/IEC 17025:2017? Y=yes, N=No, P=partly
Extr T1/T2	DNA extraction method for T1 or T2: C%=CTAB 1% or 2%; MwC=Maxwell with CTAB buffer; NS=NucleoSpin Food; NSP=NucleoSpin Plant; GS=GeneSpin; W=Promega Wizard; MF=DNeasy Mericon Food (+H=prior hexane extraction); BFP=Biotecon Foodproof; SDS=SDS method; Dex=Eurofins DNAextractor cleaning column; QE=Qiagen Qiaex II; Qq=Qiagen Qiaquick; NSc=NucleoSpin gDNA clean-up; SF(A)=SureFood Prep (Advanced); NMF=NucleoMag Food (Macherey-Nagel); NMP=NucleoMag Plant (Macherey-Nagel); EZ1=BIOROBOT EZ1 system; innuP=innuPREP Plant DNA I; FE=GeneMatrix Food Extract DNA purification kit EURx; DBT=DNeasy Blood & Tissue kit; O=Other (not specified)
Mass	Mass of powder used for DNA extraction: 1=100-199 g; 2=200-299 g; 3=300-499 g; 5=500 g or more (if different for T1 and T2, 2 values are shown)
Inh	Inhibition analysis: D=using two or more dilutions; REF or GM=prior inhibition test done for reference or GM target; PC=inclusion of internal positive control DNA; N=not tested for inhibition
73496 to Oth	Results of event-specific or screening tests: A=absent; P=present; ?=not tested or not clear; Oth=other tests (empty cell means that information is not provided)
QnT1/QnT2	Quantification approach used for T1 or T2: SC=standard curve; dCq=delta Cq; DP=digital PCR; na=not applicable (no quantification done)
CF DP	Conversion factor applied to dPCR results (in case of two values: first value is for T1, second value is for T2)
Cal	Calibrant used (in case of two different CRM producers: first is for T1, second is for T2); AOCS and JRC refer to the CRM producer; DNA means genomic DNA from the EURL; PT means proficiency test material; kit means that the calibrant was provided as part of the commercial kit
REF T1/T2	Reference gene target for T1 or T2 (in case of lectin, the number refers to the amplicon size)
CNL	Compliant under Regulation 1829/2003 and not requiring labelling as GMO
NCL	Non-compliant under Regulation 1829/2003 and requiring labelling as GMO
C619	Compliant under Regulation 619/2011 (technical threshold of 0.1 m/m % for feed)
NC619	Non-compliant under Regulation 619/2011 (technical threshold of 0.1 m/m % for feed)
CNC	Cannot conclude on compliance

Lab	T1	T2	Accr	Extr T1	Extr T2	Mass	Inh	73496	GT73	MON88302	MS8	Rf3	T45	p35S	tNOS	PAT	BAR	CP4	CTP	CTP2	Crybc	Cryb	pFMV	pNOS	t35S	nptII	35S-PAT	tE9	Oth	QnT1	QnT2	CF DP	Cal	REF T1	REF T2	CNL	NCL	C619	NC619	CNC
L01	Y	Y	P	C2%/MF+H	C2%	2	D	A	A	P	?	?	?	P	P	?	A	?	?	P	?	?	P	?	?	?	A	?	P	SC	SC		AOCS	CruA	lec74	T1	T2			
L02	Y	Y	Y	O	O	2	D	A	A	P	A	A	A	A	A	A	A	?	?	P	A	?	P	?	?	A	?	?	A	SC	SC		AOCS	Ccf	lec74	T1;T2				
L03																																							
L04	Y	Y	Y	NSF	NSF	2	GM	A	A	P	A	A	A																	SC	SC		AOCS	CruA	lec74	T1	T2			
L05	N	Y	Y		C2%+NMP	5	N	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?		DP	1.2	-		lec74	T2				
L06	Y	Y	Y	NSF	NSF	2	REF	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	A	SC	SC		AOCS	Ccf	lec74	T1;T2				
L07	Y	Y	Y	SDS/W	SDS/W	2	REF/D	A	A	P	?	?	?	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?	P	SC	SC		AOCS	Ccf	lec74	T1;T2				
L08	Y	Y	P	C2%+QE	C2%+QE	2	D	A	A	P	?	?	?	?	?	A	A	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		DNA/AOCS	CruA	lec74	T1				T2
L09	Y	Y	Y	MF	MF	2	D	?	A	?	?	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	na			AOCS	CruA	lec74	T1	T2			
L10	Y	Y	P	NSF	NSF	2	REF	A	A	P	A	A	A	P	P	A	?	?	?	?	P	?	P	?	?	?	?	?	?	SC/DP	SC/DP	0.96/0.95	AOCS	CruA/FatA(A)	lec74	T1;T2				
L11	Y	Y	Y	MwC	MwC	5	REF/GM	?	A	P	?	?	?	P	P	A	A	?	?	P	?	?	P	?	?	A	?	P	P	DP	DP	0.96/0.95	-	FatA(A)	lec74	T1;T2				
L12	Y	Y	N	NMF	NMF	2	PC	A	A	P	A	A	A	P	P	A	A			P	P		P	?	?	?	?	P		dCq	SC		AOCS	Ccf	lec74	T1;T2				
L13	N	Y	N		C2%+Qq	2	D																							SC			AOCS		lec74	T2				
L14	Y	Y	Y	NSF	NSF	2	D	A	?	P	A	A	A																	SC	SC		AOCS	CruA	lec74	T1	T2			
L15	Y	Y	Y	NSF/GS	C2%/NSF	2	REF	A	A	P	A	A	A	P	P	?	A	?	?	P	?	?	?	A	?	?	A	?	?	SC	DP	0.96	AOCS	FatA(A)	lec74	T1;T2				
L16	Y	Y	P	C2%	C2%	3/2	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		AOCS	CruA	lec74	T1;T2		T1;T2		
L17	Y	Y	P	NSF	NSF	2	O	A	A	P	A	A	A	P	P	?	A		?	P	P	?	?	?	?	?	A	?	?	SC	SC		AOCS	CruA	lec80	T1;T2				
L18																																							
L19	Y	Y	Y	MwC	MwC	1+2	REF/GM/D	A	A	P	A	A	A	P	P	A	A			P			P							SC	SC		AOCS	Ccf	lec74	T1	T2			
L20	Y	Y	Y	C2%	GS	2	REF	A	A	P	?	?	?	P	P	A	A	P	?	?	?	?	P	A	?	?	?	?		na	SC		AOCS	FatA(A)	lec74	T1	T2			
L21	Y	Y	Y	NSP	NSP	2	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	SC	DP	0.54	AOCS	CruA	lec74	T1	T2			
L22	Y	Y	N	C2%	NSF	2	REF/PC							P	P								P							na	SC		kit		lec74		T2			
L23	Y	Y	Y	SFA	SFA	2	REF/D	A	A	P	?	A	?	?	?	?	A	?	?	?	?	?	?	?	?	?	A	?		na	SC		AOCS	PEPC	lec74	T2				T1
L24	Y	Y	Y	GS	GS	2	D	A	A	P	A	A	A	A	A	A	A	?	?	P	?	?	P	?	?	?	?	?	?	SC	SC		AOCS	FatA(A)	lec74	T1;T2				
L25	Y	Y	Y	NSF	NSF	2	REF/GM	A	A	P	A	A	?	P	P	A	?	?	?	?		P	?	?	?	?	?	A	SC	SC		AOCS	Ccf	lec74	T1;T2				
L26	Y	Y	Y	C2%	C2%	2	D	A	A	P	A	A	A	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?	?	SC	SC		JRC/AOCS	FatA(A)		T1;T2				
L27	Y	Y	P	C2%	C2%/MwC	5	D	A	A	P	A	A	A	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?	P	SC	DP	0.967	AOCS	PEPC	lec74	T1				T2
L28	Y	Y	Y	NSF	NSF	5	N	A	A	P	?	?	?	P	P	?	A	?	?	P	P	?	P	?	?	?	A	?	P;A	SC	SC		AOCS	CruA	lec74		T1;T2			
L29	Y	Y	Y	C2%/MF	MF/BF	5	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	A	SC	SC		AOCS	CruA	lec74	T1;T2				
L30	Y	Y	Y	NSF	NSF	2	N	A	A	P	A	A	A	P	P	A	A	?	?	?	P		P	?	?	?	?	P	?	SC	SC		AOCS	Ccf	lec74	T1;T2				
L31	Y	Y	Y	C2%/MwC	C2%/MwC	5	D/PC	A	A	P	A	A	A	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?		SC	SC		AOCS	Ccf	lec74	T1;T2				
L32	Y	Y	P	NMF	NMF	5	REF/D	A	A	P	A	A	A	P	P	A	A	?	?	P	?	?	P	?	?	?	A	?	?	DP	DP	0.96/0.95	-	CruA	lec82	T1				T2
L33	Y	Y	Y	C2%	C2%	5	D	?	?	?	A	A	A	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?		na	DP		-	PEPC	lec74		T2			T1
L34	Y	Y	Y	MwC	MwC	2	REF	A	A	P	A	A	?	P	P	A	A	P	?	P	?	?	P	A	?	A	A	?	A	DP	DP	0.96/0.95	AOCS	Ccf	lec74	T1;T2				
L35	Y	Y	Y	MwC	MwC	5/3	REF/D/PC	A	A	P				P	P		A			P							A			SC	SC		AOCS	PEPC		T1	T2			
L36	Y	Y	Y	DNP	EZ1	2	D	?	A	P	A	A	?	P	A	A	A	?	?	P	?	?	P	?	?	?	?	P		SC	SC		AOCS	Ccf	lec74	T1				T2
L37	Y	Y	P	C2%	C2%	2	N	A	?	P	A	A	A	P	P	A	A	P	?	?	P	?	?	?		?	?	P	?	dCq	dCq		AOCS	CruA	lec74	T2				T1
L38	Y	Y	Y	MwC	MwC	1/2	REF	A	A	P	A	A	A	P	P	?	?	?	?	?	?	?	?	?	?	?	?	P	A	SC	SC		AOCS	FatA(A)	lec74	T1;T2				
L39	Y	Y	P	NSF	NSF	1	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		AOCS	CruA	lec74	T1	T2			
L40	Y	Y	Y	BF	BF	5	REF	A	A	P	?	A	?	P	P	A	A	?	?	P;?	?	?	?	?	?	?	A	A	P	SC	SC		AOCS	CruA	lec74	T1				T1;T2
L41	Y	Y	P	NSF	NSF	2	D	A	A	P				P	P	A	A	?	?	P	P	?	?	?	?	?	A	?		SC	SC		AOCS	CruA	lec74	T1;T2				
L42	Y	Y	P	innuP	innuP	2	REF/D	?	A	P	?	?	?	P	P	A	A	?	?	P	?	?	?	?	?	?	?	?	A	SC	SC		PT/AOCS	Ccf	lec74	T1;T2				
L43	Y	Y	Y	SDS/W	SDS/W	5	D	A	A	P	A	A	A	P	P	?	A	?	?	P	?	?	?	?	?	?	A	?	?	SC	SC		AOCS	Ccf	lec74	T1	T2	T1;T2		
L44	Y	Y	Y	C1%	C1%	2	REF	A	?	?	?	?	?	P	P	A	A	?	?	P			P			A				SC	SC		AOCS		lec74		T2			
L45																																							
L46	Y	Y	Y	GS	GS	2	D	A	A	P	?	?	?	P	P	A	?	?	?	P	P	?	?	?	?	?	?	P	?	na	SC		AOCS		lec74	T1;T2				
L47	Y	Y	Y	MwC+NSc	MwC+NSc	2	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		AOCS	Ccf	lec74	T1;T2				
L48	Y	Y	Y	C2%	C2%	2	REF/D	A	A	P	A	A	A	P	P	A	A	?	?	P	?	?	P	?	?	A	?	?	?	SC	SC		AOCS	CruA	lec74	T1	T2			
L49	Y	Y	Y	NSF	NSF	2	D	A	A	P	?	?	?	?	A	?	A	?	?	P	?	?	?	?	?	?	A	?	A	na	DP	0.95	AOCS		lec74	T1;T2				
L50	Y	Y	P	NSF	NSF	1	PC	A	A	P	A	A	A	P	P	?	A	?	?	P	P	?	?	?	?	?	A	?	P	SC	SC		AOCS	Ccf	lec74	T1;T2				
L51	Y	Y	Y	SF	W	2/5	REF/D	A	A	P	?	?	?	P	P	A	A	?	?	?	?	?	?	?	?	?	?	P		SC	SC		AOCS	CruA	lec74	T1	T2			
L52	Y	Y	Y	MwC	MwC	1	D	A	A	P	A	A	A	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		AOCS	CruA	lec74	T1;T2				
L53	N	Y	Y		FE	1	REF/D																							SC	SC		AOCS			T2				
L54	Y	Y	P	C2%	C2%	2	REF	A	A	P	A	A	?	P	P	A	A	?	?	P	?	?	P	A	?	?	?	P	A	SC	SC		AOCS	CruA	lec105	T1;T2				
L55	Y	Y	Y	NSF	NSF	2	D	?	?	?	?	?	?	P	P	A	A	?	?	P	?	P	?	?	?	?	?	?		na	SC	0.95	AOCS		lec74	T2		T2			
L56	Y	Y	P	O	O	2	N	A	A	?	A	A	A	P	P	?	?	?	P	?	?		P	?	?	?	?	?	A	na	SC		AOCS		lec74					T1;T2
L57	N	Y	N		C2%	2	REF/GM/D																							SC			AOCS		lec74	T2				
L58																																							
L59	Y	Y	Y	DBT	DBT	2	D	A	A	P	?	?	A	?	?	A	A	?	?	?	?	?	?	?	?	?	?	?	?	SC	SC		AOCS	CruA	lec74	T1;T2				
L60	Y	Y	Y	GS+Dex	GS+Dex	2	D	?	A	P	A	A	A	P	P	?	?	?	?	?	?	?	P	?	?	?	?	?	A	SC	SC		AOCS	Ccf	lec74					
L61	Y	Y	N	C2%	C2%	2	D	?	A	P	A	A	A	P	P	?	A			P	?	?	?	?	?	?	A	?	?	na	SC		AOCS		lec74	T2				T1
L62	Y	Y	P	GS+Dex	GS+Dex	2	D	?	A;?	P	A	A	A	P	P	?	?	?	?	?	?	?	P	?	?	?	?	?		SC	SC		AOCS	Ccf	lec74		T1;T2			
L63	Y	Y	Y	NSF/W	NSF/W	2	REF	?	A	P	A	A	A	P	A	A	A	P	?	A	A	?	A	?	?	A	?	A	?	dCq	dCq		AOCS	CruA	lec74	T1	T2		T1;T2	
L64	Y	Y	Y	C2%	C2%	1	D	A	A	P	?	?	?	P	P	A	A	?	?	P	?	?	P	?	?	A	?	?	?	SC	SC		AOCS	Ccf	lec74	T1	T2			

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