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JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS REPORT

Determination of GM Maize Events NK603 and 4114 in Maize Tortilla Chips and Maize Flour

EURL GMFF Proficiency Testing Report GMFF-19/01

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268-PT Accredited by the
Belgian Accreditation Body (BELAC)

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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-19/01) for the determination of GMOs in food and feed materials to support Regulation (EU) 2017/625 on official controls [1]. This proficiency test was open to National Reference Laboratories (NRLs) and official control laboratories (OCLs).

Two proficiency test items were distributed to participants to assess the efficacy of GMO analysis in maize tortilla chips (T1) and in maize flour (T2). T1 consisted of ground, home-made tortilla chips spiked with GM maize event NK603 (MON-ØØ6Ø3-6). T2 was composed of ground maize kernels containing GM maize event 4114 (DP-ØØ4114-3). The homogeneity and stability of the test items were evaluated and the assigned values were derived from the results reported by expert laboratories.

Fifty-three NRLs from 24 Member States and 20 OCLs participated to the exercise, comprising a total of 73 participants.

Laboratory results were rated using *z* and zeta (ζ) scores in accordance with ISO 13528:2015. No $\log_{10}$-data transformation of the reported results was applied, in line with Cordeiro *et al.*, 2019 [2]. A relative standard deviation for proficiency assessment (σ_{pt}) of 25 % was set for the two GM events, based on the experience in previous PT rounds.

The results reported indicate that a majority of participants identified the correct GM events in both test items, while using a variety of screening markers. Up to 75 % and 95 % of the reported results were satisfactory (according to the *z* score) for the quantitation of the NK603 and 4114 maize GM events in test items 1 and 2, respectively. Furthermore, most of the laboratories reported realistic measurement uncertainties. These results confirm that most control laboratories are able to determine mass fractions of GMOs in the frame of Regulation (EU) 2017/625.

Based on the reported results T1 was generally found to be 'not compliant' with the EU legislation unless labelled as containing GMO, in line with Regulation (EC) No 1829/2003 [3], while T2 was not compliant under Regulation (EU) No 619/2011 [4]. However, the correct interpretation of the labelling requirements demands further attention from some laboratories.

Finally, the EURL GMFF strongly recommends that the use of the maize reference gene target *adh1*-70 bp shall be banned for all GMO measurements by all testing laboratories.

List of abbreviations and symbols

bp	Base pairs
DG SANTE	Directorate General for Health and Food Safety
EC	European Commission
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"
$U(x_{pt})$	expanded uncertainty of the assigned value
x_i	mean value reported by participant "i"
x_{pt}	assigned value
z	z score
ζ	zeta 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 GM maize event NK603 in maize tortilla chips and GM maize event 4114 in maize flour, to support Regulation (EU) 2017/625 on official controls [1].

This PT was agreed with the Directorate General for Health and Food Safety (DG SANTE) as part of the EURL GMFF annual work programme for 2019, thus complying with the mandate set in Regulation (EU) 2017/625. The PT round was open to National Reference Laboratories under Regulations (EU) 2017/625 (NRL/625) and (EU) No 120/2014 (NRL/120) [5] and to official control laboratories (OCLs).

This report summarises 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 food and feed products. The PT was mandatory for the NRL/625, recommended for NRL/120, and open to OCLs (after formal acceptance). 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 [4].

This PT round was organised in line with ISO 17043:2010 [6] and is identified as "GMFF-19/01" (previously identified as EURL-GMFF-PT-01/19).

3 Set up of the exercise

3.1 Quality assurance

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

- ISO/IEC 17025:2005 (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 PT rounds 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-01/19 round was announced by invitation letters to NRLs and a few accepted non-EU OCLs on February 28, 2019 (Annex 1). The registration deadline was set to March 15, 2019. Registration was confirmed by email to the participants on March 29, 2019 and new deadlines for dispatch and reporting were

communicated (Annex 2). Samples were sent to participants on April 24, 2019. The deadline for reporting of results was set to June 7, 2019.

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. 1 g of powder;
- One "Test item accompanying letter" (Annex 3).

Samples were sent under room temperature conditions with a temperature probe (38 °C) attached to the accompanying letter to monitor the highest temperature to which the samples were exposed during dispatch; discoloration of the probe was not signalled by any participant.

3.5 Instructions to participants

Detailed instructions were given to participants in the "Test item accompanying letter" mentioned above.

Participants were requested to perform the following analyses:

Test Item 1: Maize tortilla chips
- Screen for the presence of any GM maize event;
- Quantify the GM event(s) detected.

Test Item 2: Maize flour (to be used as feed)
- Screen for the presence of the following three GM maize events: 1507, 4114, 59122;
- Quantify the GM event(s) detected.

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

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 and to report their measurement results. The reporting tool also allowed selecting "present", "absent" or "don't know" for GM events that were screened for but not quantified.

Participants were asked to fill in an online questionnaire through EU Survey, accessible via a password. The latter was designed to collect additional information related to the measurements and the laboratories.

4 Test item

4.1 Preparation

Test item T1 consisted of dried and ground maize tortilla chips, prepared in-house from 'masa harina' maize meal and NK603 maize (Table 1). The dough was made by adding 1.6 mL of water to the 'masa harina' and NK603 maize powders (Table 1) and then kneaded. The dough was left for 30 min to rest, then a tortilla press was used to form pancakes with uniform thickness. The pancakes were baked in a frying pan. After cooling

down, the pancakes were spread with sunflower oil, cut up into triangles of tortilla chips, and baked in an oven at 160 °C for 10 min. Following baking, the triangles were broken up by hand, frozen in liquid nitrogen and ground using a vibrating Palla-mill cooled with liquid nitrogen. The ground powder was then sieved over a 500 µm sieve and vacuum dried to reduce the water content to below 5 %. The powder was then homogenised in a three-dimensional mixer (DynaMIX CM-200) for 1 h, analysed for its water content, and approximately 5 g portions were bottled into 20 mL vials. The final T1 material had a water content of 2.0 % ± 0.3 m/m % (k=2, n=3).

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

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

Characteristic	Masa harina maize meal	NK603 maize
Type of base material	Flour	Ground maize
Origin	Naturelo, Mexico	100 % NK603 maize used for CRM production ERM-BF415
Mixing method	DynaMIX 200	
Water content in g/100 g, mean ± *U* (*k*=2, *n*=3)	8.6 ± 1.1	2.4 ± 0.3
Particle diameter in µm[1], mean ± *U* (*k*=2, *n*=3)	97.6 ± 17.3	141.7 ± 25.1
DNA yield in ng/mg[2], mean ± SD (after milling)	11.0 ± 1.9 (n=3)	83.1 ± 2.0 (n=2)
Genetic elements detected (Cq value)[3]	*hmg* (20.44)	*hmg* (22.44)
GM events detected (Cq value)[4]	MON88017 (37.05)	NK603 (23.85), MON810 (37.89)
Mass used to prepare T1 (g)	1300.02	18.56

[1] Average particle diameter of the X_{50} size class on the cumulative volume distribution curve.
[2] Results reported here for a sample intake of 200 mg with the in-house validated CTAB method. DNA yield was measured fluorometrically
[3] As these were pure maize materials the *hmg* Ct values are taken from the GM maize event-specific pre-spotted plate (PSP)
[4] GM maize event-specific pre-spotted plate (PSP) was used for these tests. Results with a Cq value above 40 are not reported
ND: Not determined; k: coverage factor; U: expanded measurement uncertainty; SD: standard deviation; n: number of replicates

The presence of different GM events in the base materials was tested using the GM maize event-specific pre-spotted plate [7]. Late positive signals for MON88017 and MON810 were detected in the 'masa harina' and NK603 samples, respectively, and they were considered to be negligible.

The amount and the quality of the DNA extracted from the T1 material were verified by UV spectrometry, fluorometry and gel electrophoresis. A CTAB method with a sample intake of 200 mg was chosen because it yielded a sufficient amount of DNA of PCR-grade quality from the base materials. However, the DNA extracted from the tortilla chips was highly degraded, as already described in [8]. The DNA extracts had to be diluted 5 times for qPCR measurements because of the large amounts of single stranded DNA (confirmed by the relatively low DNA concentration measured by PicoGreen, while the spectrophotometrically determined concentration was much higher). The calibrant, positive control and tortilla chips DNA extracts did not show any PCR inhibition when the maize *hmg* PCR assay was performed (5 µL per PCR) using DNA with a concentration diluted from 20 ng/µL to 0.08 ng/µL.

DNA extracted with a Nucleospin method was also tested for PCR inhibition on dilutions from 40 ng/µL to 0.16 ng/µL with a *hmg* qPCR assay (5 µL per PCR) and did not show any inhibition (ΔCq values were very close to the theoretical ΔCq values).

The level of fragmentation of the DNA samples extracted by the CTAB method from the T1 material was investigated by 0.8 % agarose gel electrophoresis (Figure 1). The DNA samples extracted from the tortilla chips material (lanes 2-4) and masa harina (lanes 5-7) appeared highly fragmented compared to the high molecular weight DNA extracted from the reference materials (lanes 8-13).

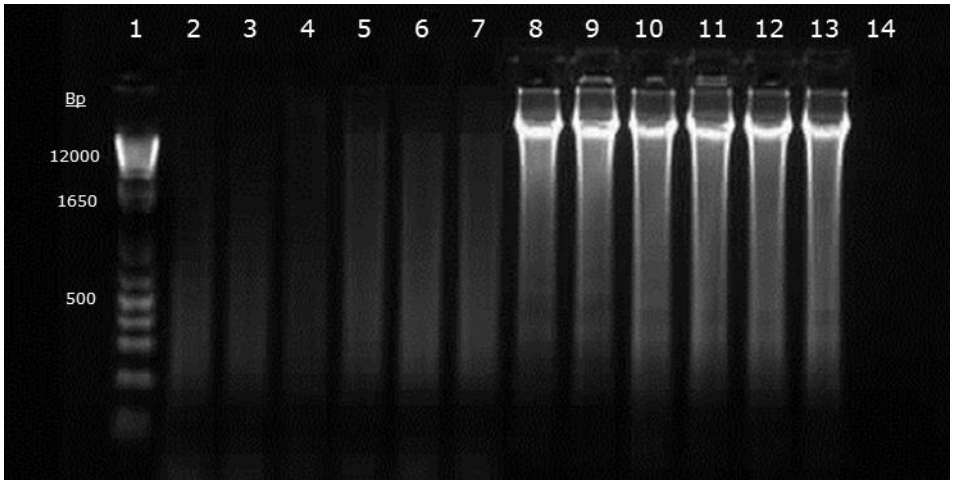

Figure 1. Agarose gel electrophoresis of genomic DNA extracted from the T1 material (lanes 2-4), masa harina (lanes 5-7) and NK603 CRMs (lanes 8-13). The blank extract is run in lane 14. The molecular marker (lane 1) is a 1 kb plus DNA ladder (Invitrogen, USA).

Test item T2 was identical to the CRM coded ERM-BF439d, certified to contain 10 g/kg DP-ØØ4114-3 maize. The PT bottles were set apart during CRM processing.

4.2 Homogeneity and stability

Measurements for the homogeneity and stability studies and the statistical treatment of data were performed for T1, using the validated event-specific detection method for NK603. Information about the homogeneity and stability of T2 were provided in the CRM certification report.

The assessment of homogeneity was performed after the preparation of the test items and before distribution to the participants. Seven bottles were randomly selected and analysed in 5 replicates. Results were evaluated according to ISO 13528:2015 [9]. The materials proved to be adequately homogeneous for the added NK603 maize (Annex 4.1). The contribution from homogeneity (u_{hom}) to the standard uncertainty of the assigned value ($u(x_{pt})$) was calculated using the software SoftCRM [10].

An isochronous short-term stability study [11] involving two test samples with three replicates each (N=2, n=3) was conducted over two weeks at +4 °C and +60 °C. The measurements by qPCR were performed under repeatability conditions. The results revealed a significant influence of 2 weeks storage at +60 °C on the stability of T1 (compared to storage at -70 °C or at +4 °C), resulting in an increase in the measured GM mass fraction. In an additional isochronous study with 4 days storage at +60 °C, a similar significant effect was observed, while storage at +4 °C or +20 °C did not reveal this effect. No effect was observed after one or two days at +40 °C or at +60 °C, followed by three or two days at room temperature, respectively. It was decided to dispatch T1 without cooling elements, and to monitor the temperature during dispatch by attaching a 38 °C temperature probe to the package. The participants were instructed to check the colour of this probe upon arrival of the package (pictures of a normal and coloured probe were provided). No change in the probe colour was reported by any participant.

The stability of T1 during the period covered by the PT was tested by qPCR, analysing the GM content in bottles (N=2, n=3) stored at the normal (+4 °C) storage temperature

during 0 and 16 weeks. The data was evaluated against storage time and a regression line was calculated. The slope of the regression line was tested for statistical significance (loss/increase due to storage). No significant trends were detected at a 95 % confidence level (Annex 4.2). This stability study confirmed that T1 was adequately stable at +4 °C during the whole period of time of the PT. The uncertainty contribution 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

The assigned value (x_{pt}) for the mass fraction of NK603 maize in maize tortilla chips was derived from results reported by JRC expert laboratories in Ispra and Geel, applying the methods validated by the EURL GMFF. The assigned values are the mean of the average values measured for four independent datasets, on DNA extracted using the CTAB DNA extraction method (3 datasets), or the Nucleospin Food kit (1 dataset). The four datasets consisted of 35 samples (*N*=7, *n*=5; "bottles" x "independent DNA extracts"; CTAB), 20 samples (*N*=4, *n*=5; CTAB), or two times 15 samples (*N*=5, *n*=3; CTAB and NucleoSpin).

The results, reported by the two expert laboratories, are presented in Table 3.

The nominal value for NK603 maize in T1 (2 %) was calculated taking into account the amount of amplifiable maize *hmg* targets extracted from the masa harina and the NK603 flour. The experimentally determined assigned value for NK603 maize (1.76 m/m %) was close to the nominal value of 2 m/m %.

For T2, the certified value of ERM-BF439d was used as assigned value (10 g/kg DP-ØØ4114-3 maize).

5.2 Associated 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 [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. Note that u_{stab} was set to zero (Annex 4.2).

5.3 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviations for PT assessment (σ_{pt}) were set for all measurands to 25 % of the respective assigned values, based on expert judgment (Table 2).

Table 2: Assigned value (x_{pt}), measurement uncertainties and standard deviation for the proficiency assessment (σ_{pt}) for T1 and T2. All values (except the last column) are expressed in m/m %

Test item	Measured average per dataset ± *U* (*k*=2)	x_{pt}	u_{char}	u_{hom}	$u(x_{pt})$	σ_{pt}	$u(x_{pt})/\sigma_{pt}$
T1	1.76 ± 0.18	**1.76**	0.05	0.09	**0.10**	**0.44**	0.23
	1.80 ± 0.39						
	1.52 ± 0.19						
	1.96 ± 0.36						
T2	NA[a]	**1.00[a]**	NA[a]	NA[a]	**0.06[a]**	**0.25**	0.22

[a] The x_{pt} and $u(x_{pt})$ were taken from the certificate of ERM-BF439d

6 Evaluation of results

6.1 Scores and evaluation criteria

The individual laboratory performance 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 5)
2 < |score| < 3	questionable performance	(yellow in Annex 5)
|score| ≥ 3	unsatisfactory performance	(red in Annex 5)

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

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

The standard measurement uncertainty of the laboratory $u(x_i)$ was obtained by dividing the reported expanded measurement uncertainty by the reported coverage factor, *k.* When no uncertainty was reported, it was set to zero ($u(x_i)$ = 0) by the PT coordinator. 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 (=1.73), as recommended by Eurachem [12].

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. $u_{max,rel}$ 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 σ_{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, in contrast to previous PT rounds organised by the EURL GMFF, reported data from participants were not $\log_{10}$-transformed prior to performance assessment [2].

6.2 General observations

Overall, 54 NRLs and 22 OCLs registered to the exercise. One OCL declined before test item dispatch, and one NRL and one OCL did not report results. The 73 participating laboratories (Table 3) represented 24 EU Member States (except Cyprus, Estonia, and Malta, while Ireland had an agreement with RIKILT, now named Wageningen Food Safety Research, in The Netherlands for GMO analysis), while 7 OCLs were from outside the EU. A total of 61 quantitative results were reported for NK603 maize and 59 results for 4114 maize. Two laboratories reported "more than" values for NK603 maize, while a few other laboratories either only reported presence/absence results for the GM events tested, *i.e.* GM event identification, or did not report any results.

The majority of participants applied real-time PCR, three applied dPCR, one OCL mentioned "nested PCR for P35S". The experimental details are provided in Annex 6.

Table 3. Overview of participants to GMFF-19/01 by country and category

Country	Participants	NRL/625	NRL/120	OCL (Not NRL)
Austria	2	2		
Belgium	3	3		
Bulgaria	2	2		
Croatia	2	1		1
Cyprus	0	0		
Czech Republic	1	1		
Denmark	1	1		
Estonia	0	0		
Finland	2	1	1	
France	3	3		
Germany	17	1	14	2
Greece	1	1		
Hong Kong	1			1
Hungary	2	1		1
Ireland	0	0		
Italy	5	1	1	3
Latvia	1	1		
Lithuania	1	1		
Luxembourg	1	1		
Malta	0	0		
Netherlands	2	1	1	
Poland	5	4		1
Portugal	1	1		
Romania	1	1		
Serbia	2			2
Slovakia	2	2		
Slovenia	1	1		
Spain	7	2		5
Sweden	1	1		
Switzerland	2			2
Turkey	2			2
United Kingdom	2	1	1	
Total	**73**	**35**	**18**	**20**

6.3 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 analysis steps. A number of OCLs are accredited only for GMO screening tests and refer the samples that are found positive for one or more of these tests to an NRL for further analysis.

NK603 maize contains the 35S promoter (P35S), Nos terminator (Tnos) and CP4-EPSPS coding sequence linked to the CTP2 transit peptide (CTP2-CP4-EPSPS). As many other common screening elements (Pat, Bar, pFMV, pNOS, nptII, tE9, cryAb[/Ac]) and screening constructs (*e.g.* CTP-CP4-EPSPS) were absent in the test item, it was not difficult to identify the NK603 event. All other maize GM events were found absent by the laboratories, except L09 who (only) found MON810 at larger than (>) 0.05 m/m %.

Among 71 participants (two participants did not analyse T1), 67 correctly identified NK603 (94 %) in T1, one failed to detect NK603 and three performed only screening analysis without final identification of the event.

- L31 applied 8 screening methods, including CP4-EPSPS that was found absent (the method would result in an imperfect annealing – see GMOmatrix on https://europa.eu/!Mu89WM), and therefore only MON87427 was retained for further analysis (this event was not tested with the event-specific method because not all reagents arrived in time);
- L06 applied 3 screening methods without further analysis among the four potential events remaining, which included NK603;
- L09 is only accredited for P35S-hsp70 screening and reported a positive result for MON810 without further analyses;
- L32 reported "absent" for NK603 in the reporting tool, but in the questionnaire, 16 potential GM maize events, including NK603, were retained on the basis of the results of 7 screening methods; although no further event-specific analysis was carried out, this participant presumably made a mistake in reporting the presence/absence of NK603 (they should have reported "don't know").

Maize event 4114 contains the P35S and Tnos regulatory elements, the PAT coding sequence, and the Cry1F and Cry34/35Ab1 insect resistance genes present also in 1507 and 59122 maize, respectively. Common screening methods would result in identical result patterns for all three events and they could only be distinguished by event-specific methods. As only these three events had to be tested, most participants directly applied the event-specific methods to T2. The reported results showed that among 71 participants (two participants did not analyse T2), 59 correctly identified the 4114 event in T2. L67 (OCL) reported "absent" in the reporting tool (no questionnaire returned), and the remaining 11 participants, including 3 NRL/625, had not analysed the 4114 event because of (i) the absence of the reagents (CRM, primers or probe) required for the method, or (ii) the method was not verified by the laboratory or (iii) the method was out of the scope of accreditation.

Note that the presence/absence dropdown list in the online reporting tool was not always carefully applied, *e.g.* "absent" was selected for a specified GM event while a quantitative result was provided in the cell on the same row. In the latter case, the result was interpreted as 'positive' for the GM event identification.

6.4 Laboratory results and scorings

6.4.1 Performances

Laboratory performance for (qualitative) identification of the GM event in a test item was scored using the following qualitative terms: D=detected, ND=not detected, NT=test item or GM event not tested. These qualitative results are not discussed below, since the PTs organised by the EURL GMFF target primarily NRLs expected to provide quantitative results.

Laboratory performance for quantitation of the GM event in a test item was scored using z and ζ scores. Satisfactory performances are highlighted in green, questionable in yellow, unsatisfactory in red. A cell was left uncoloured when no quantitative result was reported.

Annex 5 presents the reported results as tables and graphs for each measurand. The corresponding Kernel density plots have been obtained by using the software available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [13].

Figure 2 summarises the performance scores obtained. Most of the laboratories (over 70 %) reported satisfactory results for the two test items.

A total of 9 unsatisfactory *z* scores were obtained for T1 and one for T2. Slightly lower rates of satisfactory performance were observed for *ζ* scores compared to *z* scores. As explained in Section 6.1, an unsatisfactory performance expressed as *ζ* score may be due to a biased results that strongly deviated from the assigned value (inducing an unsatisfactory *z* score) or it may indicate an underestimation of the measurement uncertainty.

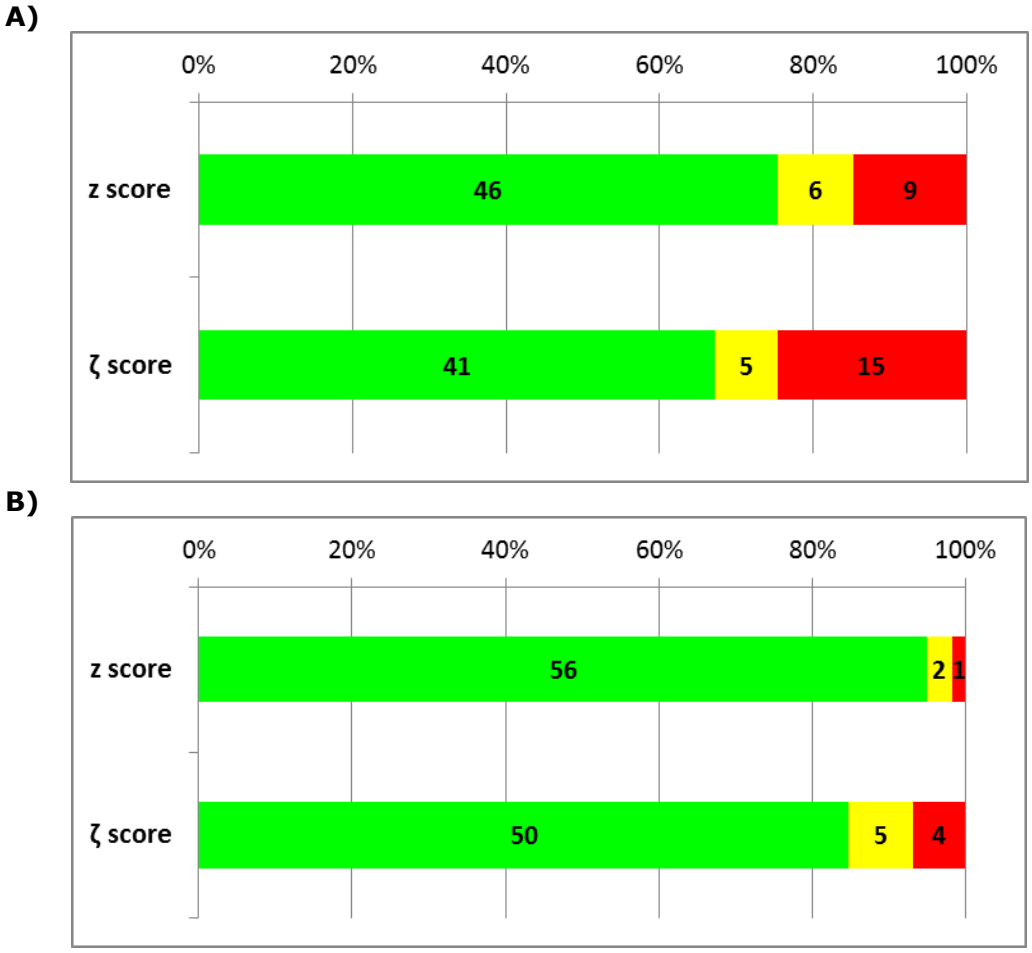

Figure 2. Overview of laboratory performance per measurand according to *z* and *ζ* scores, for the content of NK603 maize in test item T1 (A) and 4114 maize in test item T2 (B). Corresponding numbers of laboratories are shown in the bars. Satisfactory, questionable and unsatisfactory performances are indicated in green, yellow and red, respectively.

6.4.2 Truncated values

Laboratories L50 and L58 reported truncated ('more than') values for NK603 maize in T1 (>1 and >0.05 m/m %, respectively). From L50 (NRL/120), the reported value was provided as indicative value only as the minimum performance criteria were not fulfilled (Cq values were outside the standard curve). For L58 (OCL), who identified the NK603 event but did not quantify it, the truncated value presumably corresponded to the limit of quantification (LOQ) or limit of detection (LOD) of the applied method (no questionnaire returned). These truncated values could not be included in the data evaluation. However, they were compared with the corresponding $x_{pt} - U(x_{pt})$. Since the reported truncated values were lower than $x_{pt} - U(x_{pt})$, the statements were considered correct.

6.4.3 Measurement uncertainties

The large majority of laboratories having reported quantitative results provided expanded measurement uncertainties and coverage factors. All NRLs reported the measurement uncertainties associated to the NK603 and 4114 results, but three (NK603) and five NRLs (4114), respectively, did not report the coverage factor applied (Annex 5). Similarly, a few OCLs did not report their measurement uncertainty or coverage factor. When no measurement uncertainty was reported, it was set to zero in the calculations in Annex 5, while a *k* factor of 1.73 was used in case the actual coverage factor was not reported.

The measurement uncertainties were evaluated according to [9] (See section 6.1), which indicated that more than 70 % of laboratories provided a realistic measurement uncertainty (Case "a" in Figure 3).

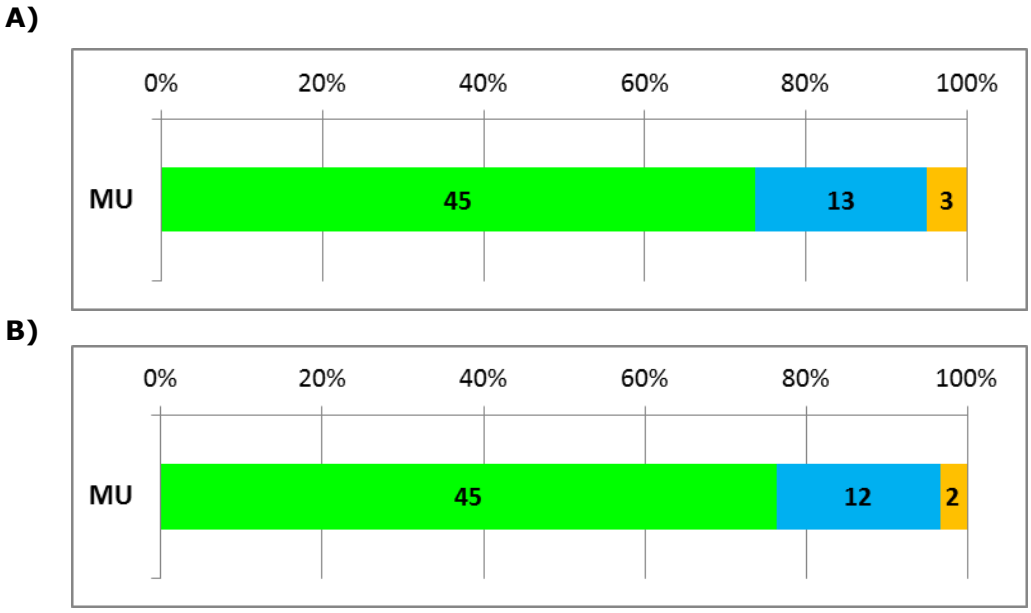

Figure 3. Review of uncertainties reported for the content of NK603 maize in test item T1 (A) and 4114 maize in test item T2 (B). Corresponding number of laboratories indicated in the graph.
Case "a" (green): $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt}$
Case "b" (blue): $u_{rel}(x_i) < u_{rel}(x_{pt})$
Case "c" (orange): $u_{rel}(x_i) > \sigma_{pt}$

6.4.4 Compliance statement

Regulation (EC) No 1829/2003 [3] established a threshold for labelling of food and feed products containing GM material that is authorised in the EU (0.9 %). Furthermore, Regulation (EU) No 619/2011 [4] introduced a minimum performance limit (0.1 m/m %) for detecting the accidental presence, in feed, of GM material with pending or expired authorisation status. Compliance with these values is verified by the Member States of the European Union during the official control of food and feed.

In this PT round, laboratories were requested to provide a compliance statement for the T1 and T2 samples, in relation to the applicable EU legislation. 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 interprete their results. The reported range (result ± expanded uncertainty) is to be compared to (i) the labelling threshold of 0.9 m/m % for authorised GMOs; or (ii) the technical threshold of 0.1 m/m %.

Sixty-four participants filled in the questions regarding compliance. An example of a correct compliance assessment is provided below for test items T1 and T2.

Test item T1

The following assumptions were taken into account:

- The GM soybean NK603 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) content range of NK603 in T1 is: 1.76 ± 0.20 (k=2) m/m %,
- this range is - beyond any reasonable doubt - above the labelling threshold, since 1.76 - 0.20 > 0.9 m/m % (x - U > Threshold).
- This material is to be considered as "**Not compliant**" considering that the sample was not labelled as GMO, or "**Compliant – requiring labelling**".

Participants were requested to choose among four compliance statements when evaluating sample T1: (i) compliant – not requiring labelling (CNL), (ii) compliant – to be labelled (C2L), (iii) not compliant (NC), or (iv) cannot be concluded. Table 4 clusters their statements, taking into account the reported analytical results (or lack of results).

For T1, the majority of the laboratories (53 out of 64 responses, *i.e.* 83 %) correctly interpreted the compliance rules based on their obtained measurement results. Most of these participants concluded that the GMO content was above 0.9 % and, therefore, the sample either required labelling (48 responses), or was considered "not compliant" (5 responses). Indeed, the accompanying letter had indicated that the test item was taken from a sample that was not labelled as containing GMOs and therefore should be considered not compliant (this information may have been overlooked by many participants). Three other laboratories, all NRLs, concluded that the sample did not require labelling because the measured content (with measurement uncertainty) was below 0.9 % (see comment in Table 4). One participant reported C2L, but the result reported minus the measurement uncertainty was below 0.9 % (due to an overestimated measurement uncertainty), hence CNL should have been concluded. Eight laboratories, including three NRLs, reported "cannot be concluded", mostly because no quantification was done. A compliance statement was not received from the remaining 9 participants.

Table 4. Reported compliance statements for T1 (food containing NK603 maize)

Compliance Statement (*)	Laboratory Measurement	Number of Laboratories	Comment
CNL	x ± U > 0.9 m/m %	0	
	x ± U ≤ 0.9 m/m %	3	L23, L45, L74 (NRL/625): correct statement, but result too low and/or measurement uncertainty too high
C2L	x ± U > 0.9 m/m %	47	**Correct**
	x ± U ≤ 0.9 m/m %	1	L72 (OCL)
NC	x ± U > 0.9 m/m %	5	**Correct** (considering that the sample was not labelled)
Cannot be concluded	Quantified	1	L37 (OCL)
	Not quantified	7	L22, L31, L32 (NRL/625) L06, L44, L54, L76 (OCL)
Total		*64*	

(*) CNL: compliant – not to be labelled; C2L: compliant – to be labelled; NC: not compliant

Test item T2

A similar evaluation of compliance assessment was done for T2, containing 4114 maize, which was labelled as feed:

- The 4114 event is not an authorised GM event in the EU, but fulfils the requirements of Regulation (EU) No 619/2011 [4], hence
- the technical threshold to be applied is 0.1 m/m %.
- Knowing that the assigned (expanded) range is 1.00 ± 0.11 (k=2) m/m %,
- this range is - beyond any reasonable doubt - above the technical threshold.
- This material is to be considered "**not compliant**".

The majority of participants (47 out of 64, *i.e.* 73 %) correctly considered the sample as not compliant (Table 5). However, only 33 of these participants selected the correct reason for non-compliance, *i.e.* that the event falls under Regulation (EU) No 619/2011 and its content was higher than 0.1 m/m %. Another 13 out of the 47 participants stated that the event was not authorised in the EU (which would be correct if the sample was food), and one NRL/625 concluded that the sample was non-compliant because it was authorised but present above 0.9 %. Seven other participants, including 5 NRL, declared the sample compliant if labelled, and one NRL/120 stated compliant and no labelling required; these statements are wrong, as the labelling rules do not apply for GM events falling under Regulation (EU) No 619/2011. A compliance statement was not received from the remaining 9 participants.

Table 5. Reported compliance statements for T2 (feed containing 4114 maize)

Compliance Statement (*)	Laboratory Measurement	Number of Laboratories	Comment
CNL	x ± U > 0.1 m/m %	0	
	x ± U ≤ 0.1 m/m %	1	L01 (NRL/120)
C2L	x ± U > 0.1 m/m %	7	L12, L33, L57, L66 (NRL/625) L43 (NRL/120) L75, L76 (OCL)
	x ± U ≤ 0.1 m/m %	0	
NC	x ± U > 0.1 m/m %	47	**Correct**
Cannot be concluded	Quantified	0	
	Not quantified	9	L32, L35, L71 (NRL/625) L06, L08, L18, L36, L44, L56 (OCL)
Total		*64*	

(*) CNL: compliant – not to be labelled; C2L: compliant – to be labelled; NC: not compliant

6.4.5 Additional information extracted from the questionnaire

The questionnaire was answered by 66 participants giving valuable information on the laboratories, their way of working and their analytical methods. Annex 6 summarises the experimental details, the technique used and the compliance declarations.

For both test items, approximately half of the participants applied a DNA extraction method involving lysis with CTAB, the other half used one of several commercial kits for DNA extraction, mostly NucleoSpin Food. Sample intake for extraction was 200 mg for most laboratories, but 16 laboratories used 500 mg or more. This resulted in a sufficient amount of DNA when analysed spectrophotometrically (often > 100 ng/µL), but the few laboratories that measured the DNA concentration with PicoGreen measured much less, indicating the presence of single-stranded DNA in the extracts. Most laboratories used screening methods to limit the number of GMOs to test by event-specific methods. The

most common strategy, used by 25 laboratories, involved testing for (at least) P35S, Tnos, PAT, BAR and ctpt2-CP4-EPSPS.

Surprisingly, 14 laboratories, including 7 NRLs, reported the use of the *adh1*-70 bp taxon-specific reference gene for quantification of NK603 maize in T1, which is unacceptable. Evidence, published already in 2008 [14], revealed that this method is unreliable and should be replaced by another maize-specific reference method, see *e.g.* warnings included in several validation reports (*e.g.* https://europa.eu/!kQ74qr) and in the NK603 method protocol in the GMOMethods database (See comment in https://europa.eu/!xw36Fm). The *adh1*-70 bp method was not used by any laboratory for quantification of 4114 maize in T2.

A number of laboratories reported problems with the efficiency of the calibration curve for NK603 (10 laboratories reported slopes below -3.6), which is a known observation for this method.

Several approaches were used to estimate measurement uncertainties. Most of the laboratories (26) derived their uncertainty estimates from measuring replicates.

7 Conclusions

The proficiency test GMFF-19/01 was organised to assess the analytical capabilities of EU NRLs and OCLs to determine the content of NK603 and 4114 maize in food (maize tortilla chips) and feed (maize flour) products, respectively.

The overall performance of the participants for the determination of these GM events was satisfactory. This confirms their analytical capabilities to enforce the Commission Regulation (EU) 2017/625, even for difficult matrices from highly processed products.

Similarly, 83 % and 73 % of the participants correctly assessed the compliance of the test item with regard to Regulations (EC) No 1829/2003 and (EU) No 619/2011, respectively. Several laboratories need to evaluate their conclusions more carefully, taking into account the authorisation status of the GM events and the measurement uncertainty contribution to the result.

The use of the *adh1*-70 bp maize-specific reference method shall be banned by all laboratories for all quantitative GMO methods.

Most of the participants reported realistic measurement uncertainty estimations.

Acknowledgements

The seventy-three laboratories listed hereafter are kindly acknowledged for their participation to the PT.

Organisation	Country
Umweltbundesamt GmbH	AUSTRIA
AGES - Institute for Food Safety Vienna	AUSTRIA
ILVO	BELGIUM
SCIENSANO	BELGIUM
Walloon Agricultural Research Center CRA-W	BELGIUM
SGS Bulgaria Ltd	BULGARIA
National Center of Public Health and Analyses	BULGARIA
Croatian Institute of Public Health	CROATIA
Croatian Agency for Agriculture and Food, Centre for Seed and Seedlings	CROATIA
Crop Research Institute	CZECH REPUBLIC
Danish Veterinary and Food Administration	DENMARK
Finnish Customs Laboratory	FINLAND
Finnish Food Authority	FINLAND
Laboratoire de la Santé des Végétaux - Anses	FRANCE
BioGEVES	FRANCE
Service Commun des Laboratoires	FRANCE
Landeslabor Schleswig-Holstein	GERMANY
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V (LALLF MV)	GERMANY
Bavarian Health and Food Safety Authority (LGL)	GERMANY
Landesuntersuchungsamt	GERMANY
Institute for Hygiene and Environment	GERMANY
Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe	GERMANY
CVUA Freiburg	GERMANY
LTZ Augustenberg	GERMANY
LUFA Speyer	GERMANY
LAVES	GERMANY
Bfr	GERMANY
Landesamt für Verbraucherschutz Sachsen-Anhalt	GERMANY
Thüringer Landesamt fuer Verbraucherschutz	GERMANY
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	GERMANY
Federal Office of Consumer Protection and Food Safety (BVL)	GERMANY
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	GERMANY
Thüringer Landesamt für Landwirtschaft und Ländlichen Raum	GERMANY
General Chemical State Laboratory	GREECE
Government Laboratory, HKSAR	HONG KONG
National Food Chain Safety Office	HUNGARY
Biomi Ltd	HUNGARY
Istituto Zooprofilattico Sperimentale Abruzzo e Molise	ITALY
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta	ITALY
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana	ITALY
CREA Centro di ricerca Difesa e Certificazione	ITALY
Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna	ITALY
Institute of Food Safety, Animal Health and Environment	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
Laboratoire National de Santé	LUXEMBOURG
RIKILT Wageningen University & Research	NETHERLANDS

Netherland Food and Consumer Product Safety Authority(NVWA)	NETHERLANDS
Plant Breeding and Acclimatization Institute NRI	POLAND
National Veterinary Research Institute	POLAND
Regional Laboratory of Genetically Modified Food	POLAND
Instytut Zootechniki PiB KLP Pracownia w Szczecinie	POLAND
Wojewodzki Inspektorat Weterynarii w Opolu	POLAND
INIAV	PORTUGAL
Institute for Diagnosis and Animal Health	ROMANIA
SP Laboratorija a.d.	SERBIA
A Bio Tech Lab	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
Laboratorio S. Pública	SPAIN
Laboratorio de Salud Pública. Madrid-Salud. Ayuntamiento de Madrid	SPAIN
Laboratori Agroalimentari	SPAIN
Laboratorio Arbitral Agroalimentario - MAPA	SPAIN
Centro Nacional de Alimentación (Agencia Española de Seguridad Alimentaria y Nutrición)	SPAIN
Laboratorio de Producción y Sanidad Vegetal de Sevilla	SPAIN
Laboratorio Agroalimentario de Cordoba	SPAIN
Livsmedelsverket (National Food Agency)	SWEDEN
Agroscope	SWITZERLAND
Food Safety and Veterinary Office FSVO	SWITZERLAND
Ankara Food Control Laboratory Directorate	TURKEY
National Reference Laboratory	TURKEY
LGC	UNITED KINGDOM
Fera	UNITED KINGDOM

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Annexes

Annex 1: Invitation letter

Ref. Ares(2019)1405558 - 01/03/2019

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 1 March 2019
JRC.F.5/HE/wb/bk/ARES(2019) 19-021

NOTE FOR THE ATTENTION OF

National Reference Laboratories (NRLs)

Subject: Invitation to participate to proficiency test EURL-GMFF-PT-01/19

Dear Colleague,

Hereby, I would like to invite you for participating to the proficiency test (PT) EURL-GMFF-PT-01/19, 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. **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, processed by the JRC, to be analysed for the presence and mass fraction of GMOs. This PT will also assess the efficiency of the screening strategies applied in the laboratory. The following tasks are requested from the participants:

Test Item 1: Maize tortilla chips (food)
- Screen for the presence of any GM maize event;
- Quantify the GM event(s) detected.

Test Item 2: Maize flour (to be used as feed)
- Screen for the presence of the maize events 1507, 4114 and 59122;
- Quantify the GM event(s) detected.

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.

The identification information on 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)

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 722. https://ec.europa.eu/jrc/
E-mail: hendrik.emons@ec.europa.eu

2017/625 will be disclosed to DG SANTE for the purpose of the evaluation of their performance. Upon request from the NRL in a Member State, the lab codes of the official laboratories 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=2202

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 15 March 2019**.

Samples will be shipped during the **week of 2 April 2019**. You are requested to inform us promptly if you have not received the samples by 12 April.

The deadline for submission of the results is **Friday 17 May 2019**.

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,

Prof Dr Hendrik Emons
Head of Unit

Contact:

European Reference Laboratory for GM Food and Feed
Dr Wim Broothaerts, Project leader GMO Control
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: Confirmation of registration

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

EURL
European Union Reference Laboratory
for GM Food & Feed

Geel, 29 March 2019
JRC.F.5/WB/mt 19-018

To: All Laboratories registered for the proficiency test EURL-GMFF-PT-01/19

Subject: Confirmation of registration

Dear Colleague,

This letter constitutes **an official proof of your successful registration** for the proficiency test EURL-GMFF-PT-01/19.

Note that the test items will be dispatched during the week after Eastern (Wednesday 24 April 2019). The deadline for submission of the results is Friday 7 June 2019.

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

Yours sincerely,

Wim Broothaerts
Project leader GMO Control
European Union Reference Laboratory for GM Food and Feed

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 722. https://ec.europa.eu/jrc/
E-mail: hendrik.emons@ec.europa.eu

Annex 3: Test item accompanying letter

Ares(2019)1814062

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 23 April 2019
JRC.F.5 WB

NOTE FOR THE ATTENTION OF

All Laboratories registered for the Proficiency test EURL-GMFF-PT-01/19

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

Subject: **EURL-GMFF-PT-01/19, a proficiency test (PT) to determine the GM content in two test materials, *i.e.* maize tortilla chips and maize flour**

Dear Dr «Surname»,

Thank you for participating to EURL-GMFF-PT-01/19. Please find in this parcel two test materials, T1 and T2, containing approximately 5 g and 1 g of ground sample, respectively. Please check whether the containers containing the test item remained undamaged during transport and promptly inform us if this is not the case. There is no need to send proof of the delivery to the EURL GMFF.

You should store the samples in a dark and cold place (at approximately 4 °C).

It is recommended to use a **minimum sample intake of 200 mg** for your DNA extractions, as homogeneity of the ground samples has been demonstrated using this amount of sample.

Tasks
Participants should perform the following analyses:

Test Item 1: Maize tortilla chips
- Screen for the presence of any GM maize event;
- Quantify the GM event(s) detected.

Test Item 2: Maize flour (to be used as feed)
- Screen for the presence of the following three GM maize events: 1507, 4114, 59122;
- Quantify the GM event(s) detected.

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.

The quantitative results, **to be reported with two decimal places (e.g. 0.64 or 1.29)**, have to be reported in mass/mass % (not accepted: copy/copy %) as outlined below:

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

Please take into account the following general rule: results obtained using a calibrant certified for GM mass fraction (*i.e.* a matrix CRM certified in [x] g/kg) can directly be expressed in m/m %, while results obtained

using a calibrant certified for DNA copy number ratio (*e.g.* a plasmid containing both the GM and reference gene target or some matrix CRMs) need to be converted into m/m %, using a conversion factor.

You are requested to pay attention to the correct estimation and reporting of the measurement uncertainty 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.

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.

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.

When entering your results, please select the correct choices from the dropdown menus, including for "Unit" and "Technique". Under "Unit" you have to select either "m/m %" if you provide a measurement result, or indicate the GM identification result for the GM event ("present", "absent", "don't know" [= not tested]). This needs to be done for all GM events in the table.

Don't forget to click the "validate and save" button, and after going back to the main page, 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, make a scanned copy and send this back 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/PT0119questionnaire, enter the password (**PT0119**), 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 7 June 2019. 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 stored at approximately 4 °C in order to voluntary repeat the analysis in case of an unsatisfactory performance. Please, dispose of the test items thereafter.

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

Thank you for the collaboration in this PT.

Yours sincerely,

Wim Broothaerts
Project leader GMO control
European Union Reference Laboratory for GM Food and Feed

Annex 4: Homogeneity and stability results

Homogeneity NK603 maize in T1

Bottle	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	1,6	1,95	2,14	1,37	1,59
40	1,87	1,55	1,91	1,68	1,62
62	1,61	1,52	1,8	1,81	1,76
110	1,85	2,24	1,96	1,79	1,63
121	1,55	1,77	1,72	1,91	1,75
150	2,16	2,14	1,94	1,56	1,89
188	1,72	1,73	1,78	1,71	1,65
Mean	1.78				
s_x	0.096				
s_w	0.195				
S_s	0.040				
u^*	0.045				
σ_{pt}	0.440				
$0.3 * \sigma_{pt}$	0.132				
$s_s \leq 0.3^* \sigma_{pt}$	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 [15]

Stability NK603 maize in T1

Weeks	Bottle no.	Replicate 1	Replicate 2	Replicate 3	Average
0	83	1.77	1.76	1.85	1.72
	172	1.66	1.68	1.62	
16	114	1.50	-	1.90	1.85
	186	2.38	1.72	1.75	

Slope ± 2 $SE_{(slope)}$ = 0.0051 ± 0.0102

Stability: Passed.

Annex 5: Results and laboratory performance

NK603 maize in T1 (all values in m/m %):
Assigned range: x_{pt} = 1.76 ± 0.20 ($U(x_{pt})$, k = 2) ; σ_{pt} = 0.44

- ID = (qualitative) performance for GM event identification: D = detected (green); ND = not detected (red); NT = not tested (no colour).
- The PT coordinator set the measurement uncertainty $u(x_i)$ to zero when no expanded uncertainty was reported, and k = 1.73 when no coverage factor (k) was reported.
- Performance scores - satisfactory (green); questionable (yellow); unsatisfactory (red).
- Measurement uncertainty (MU) - a: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt}$; b: $u_{rel}(x_i) < u_{rel}(x_{pt})$; c: $u_{rel}(x_i) > \sigma_{pt}$
- Compl. = Compliance statement: CNL: compliant not to be labelled; C2L: compliant to be labelled; NC: not compliant (green, correct statement); "?": undecided; "--" no answer.

Lab Code	Type	ID	x_i	±	k	$u(x_i)$	z score	ζ score	MU	Compl.
L01	NRL/120	D	1,45	0,29	2,00	0,145	-0,7	-1,8	a	C2L
L02	NRL/120	D	1,22	0,18	2,20	0,082	-1,2	-4,2	a	C2L
L03	NRL/625	D	3,60	0,85	2,00	0,425	4,2	4,2	a	C2L
L04	NRL/120	D	2,46	0,46	2	0,230	1,6	2,8	a	C2L
L05	NRL/625	D	1,94	0,52	2,00	0,260	0,4	0,6	a	C2L
L06	OCL	NT								?
L07	NRL/120	D	1,71	0,33	2,23	0,148	-0,1	-0,3	a	C2L
L08	OCL	D	7,00			0,000	11,9	52,0	b	C2L
L09	OCL	NT								--
L10	NRL/625	D	1,83	0,34	2,00	0,170	0,2	0,4	a	C2L
L11	NRL/120	D	0,90	0,20	2,00	0,100	-2,0	-6,1	a	--
L12	NRL/625	NT								--
L13	NRL/625	NT								--
L14	OCL	D	2,38	0,83	2,00	0,415	1,4	1,5	a	NC
L15	NRL/625	D	3,78	0,27	2,00	0,135	4,6	12,0	b	C2L
L16	NRL/625	D	1,64	0,32	2,00	0,160	-0,3	-0,6	a	C2L
L17	OCL	D	1,91	0,40	2,00	0,200	0,3	0,7	a	C2L
L18	OCL	D	5,69	1,97	2,00	0,985	8,9	4,0	a	C2L
L19	NRL/120	D	1,59	0,56	2,00	0,280	-0,4	-0,6	a	C2L
L20	NRL/625	D	1,55	0,23	2,00	0,115	-0,5	-1,4	a	C2L
L21	NRL/625	D	1,79	0,42	2,00	0,210	0,1	0,1	a	C2L
L22	NRL/625	D								?
L23	NRL/625	D	1,44	0,72	2,00	0,360	-0,7	-0,9	a	CNL
L24	NRL/120	D	2,11	0,14	2,00	0,070	0,8	2,9	b	C2L
L25	NRL/625	D	1,53	0,38	2,00	0,190	-0,5	-1,1	a	C2L
L26	NRL/625	D	2,45	0,69	2,00	0,345	1,6	1,9	a	C2L
L27	NRL/625	D	5,52	0,25	1,73	0,145	8,5	21,3	b	C2L
L28	NRL/625	D	3,25	0,63	2,00	0,315	3,4	4,5	a	NC
L29	NRL/625	D	1,84	0,47	2,00	0,235	0,2	0,3	a	C2L
L30	NRL/625	D	1,65	0,12	4,30	0,028	-0,3	-1,1	b	C2L
L31	NRL/625	ND								?
L32	NRL/625	NT								?
L33	NRL/625	D	2,30	0,74	2,00	0,370	1,2	1,4	a	C2L
L34	NRL/120	D	1,75	0,28	1,60	0,175	0,0	0,0	a	C2L
L35	NRL/625	D	3,03	0,90	2,00	0,450	2,9	2,8	a	C2L
L36	OCL	D	1,57			0,000	-0,4	-1,9	b	NC
L37	OCL	D	3,29	1,18	2,00	0,590	3,5	2,6	a	?
L38	OCL	D	6,04			0,000	9,7	42,5	b	C2L
L39	NRL/625	D	2,68	0,98	1,73	0,566	2,1	1,6	a	NC
L40	NRL/120	D	2,00	0,60	3,18	0,189	0,5	1,1	a	C2L

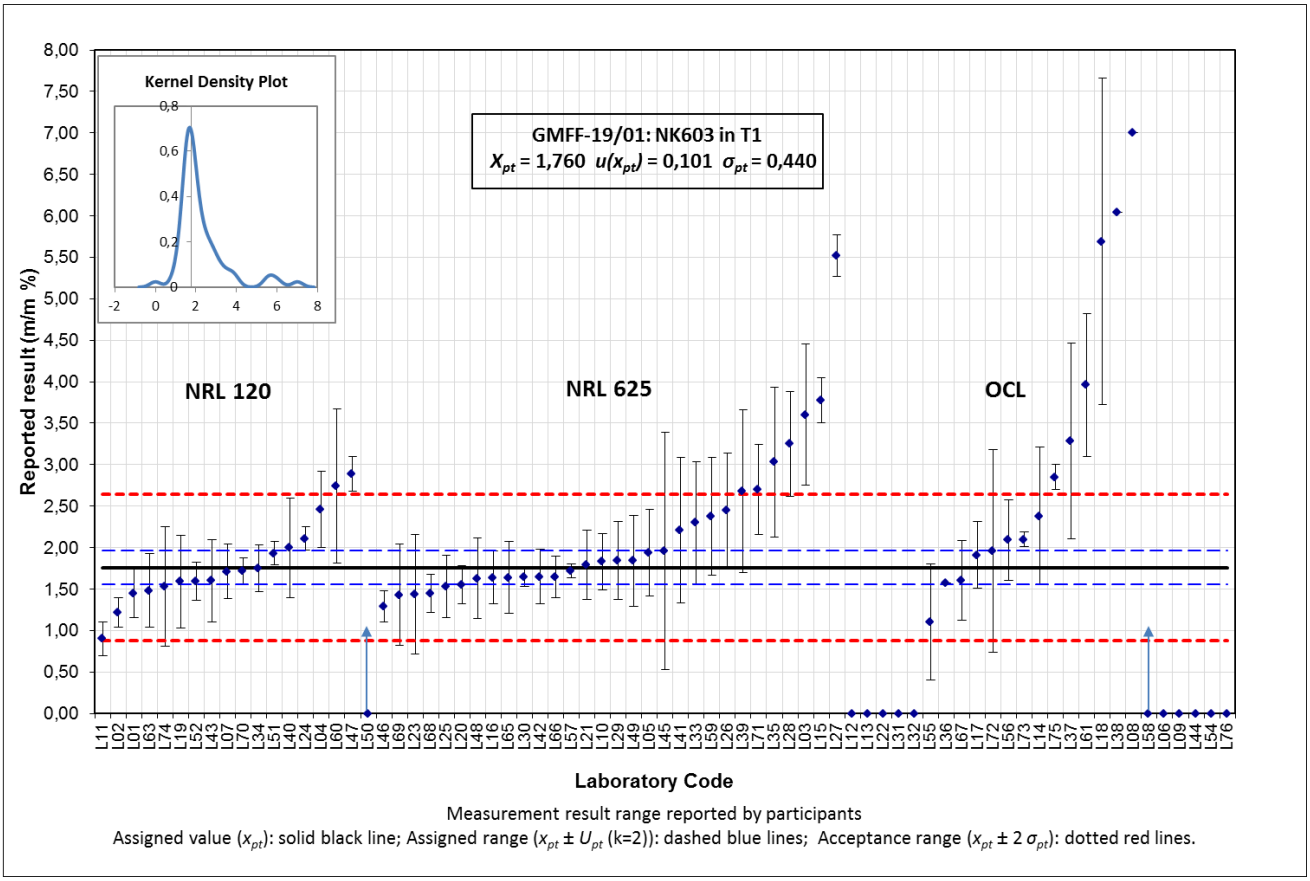
Lab Code	Type	ID	x_i	±	k	$u(x_i)$	z score	ζ score	MU	Compl.
L41	NRL/625	D	2,21	0,88	2,00	0,440	1,0	1,0	a	NC
L42	NRL/625	D	1,65	0,33	2,13	0,155	-0,3	-0,6	a	C2L
L43	NRL/120	D	1,60	0,50	2,18	0,229	-0,4	-0,6	a	C2L
L44	OCL	D								?
L45	NRL/625	D	1,96	1,43	2,00	0,715	0,5	0,3	c	CNL
L46	NRL/625	D	1,29	0,19	2	0,095	-1,1	-3,4	a	C2L
L47	NRL/120	D	2,89	0,21	2,00	0,105	2,6	7,8	b	C2L
L48	NRL/625	D	1,63	0,49	2,00	0,245	-0,3	-0,5	a	--
L49	NRL/625	D	1,84	0,55	2,00	0,276	0,2	0,3	a	C2L
L50	NRL/120	D	> 1							C2L
L51	NRL/120	D	1,93	0,14	2,00	0,070	0,4	1,4	b	C2L
L52	NRL/120	D	1,59	0,23	2,00	0,115	-0,4	-1,1	a	C2L
L54	OCL	D								?
L55	OCL	D	1,10	0,70	1,73	0,405	-1,5	-1,6	c	--
L56	OCL	D	2,09	0,49	2,00	0,245	0,8	1,2	a	C2L
L57	NRL/625	D	1,72	0,09	2,00	0,043	-0,1	-0,4	b	C2L
L58	OCL	D	> 0,05							--
L59	NRL/625	D	2,38	0,71	1,73	0,410	1,4	1,5	a	C2L
L60	NRL/120	D	2,74	0,93	2,00	0,465	2,2	2,1	a	C2L
L61	OCL	D	3,96	0,86	2,00	0,430	5,0	5,0	a	C2L
L63	NRL/120	D	1,48	0,44	2,00	0,222	-0,6	-1,1	a	C2L
L65	NRL/625	D	1,64	0,43	2,23	0,193	-0,3	-0,6	a	C2L
L66	NRL/625	D	1,65	0,25	2,00	0,125	-0,3	-0,7	a	C2L
L67	OCL	D	1,60	0,48	2,00	0,240	-0,4	-0,6	a	--
L68	NRL/625	D	1,45	0,23	2,00	0,115	-0,7	-2,0	a	C2L
L69	NRL/625	D	1,43	0,61	2,00	0,305	-0,8	-1,0	a	--
L70	NRL/120	D	1,72	0,16	2,57	0,062	-0,1	-0,3	b	C2L
L71	NRL/625	D	2,70	0,54	2,00	0,270	2,1	3,3	a	C2L
L72	OCL	D	1,96	1,22	2,00	0,610	0,5	0,3	c	C2L
L73	OCL	D	2,10	0,09	1,73	0,049	0,8	3,0	b	C2L
L74	NRL/120	D	1,53	0,72	2	0,360	-0,5	-0,6	a	CNL
L75	OCL	D	2,85	0,15	2	0,075	2,5	8,7	b	C2L
L76	OCL	D								?

4114 maize in T2 (all values in m/m %):
Assigned range: x_{pt} = 1.00 ± 0.11 ($U(x_{pt})$, k = 2) ; σ_{pt} = 0.25

- ID = (qualitative) performance for GM event identification: D = detected (green); ND = not detected (red); NT = not tested (no colour).
- The PT coordinator set the measurement uncertainty $u(xi)$ to zero when no expanded uncertainty was reported, and k = 1.73 when no coverage factor (k) was reported.
- Performance scores - satisfactory (green); questionable (yellow); unsatisfactory (red).
- Measurement uncertainty (MU) - a: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt}$; b: $u_{rel}(x_i) < u_{rel}(x_{pt})$; c: $u_{rel}(x_i) > \sigma_{pt}$
- Compl. = Compliance statement: CNL: compliant not to be labelled; C2L: compliant to be labelled; NC: not compliant (green, correct statement); "?": undecided; "--" no answer.

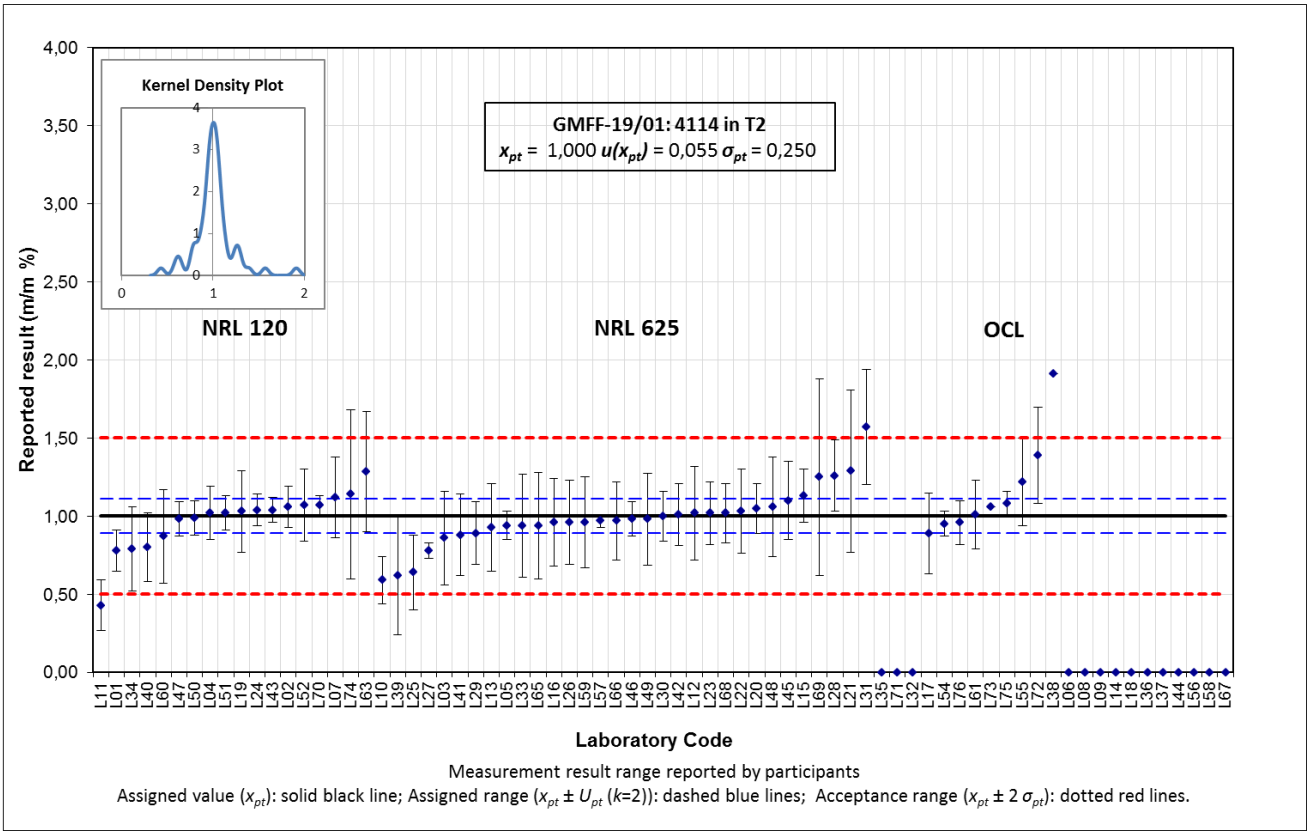
Lab Code	Type	ID	x_i	±	k	$u(x_i)$	z score	ζ score	MU	Compl.
L01	NRL/120	D	0,78	0,13	2,00	0,065	-0,9	-2,6	a	CNL
L02	NRL/120	D	1,06	0,13	2,20	0,059	0,2	0,7	a	NC
L03	NRL/625	D	0,86	0,30	2,00	0,150	-0,6	-0,9	a	NC
L04	NRL/120	D	1,02	0,17	2,00	0,085	0,1	0,2	a	NC
L05	NRL/625	D	0,94	0,09	2,00	0,045	-0,2	-0,8	b	NC
L06	OCL	NT								?
L07	NRL/120	D	1,12	0,26	2,00	0,130	0,5	0,9	a	NC
L08	OCL	NT								?
L09	OCL	NT								--
L10	NRL/625	D	0,59	0,15	2,00	0,075	-1,6	-4,4	a	NC
L11	NRL/120	D	0,43	0,16	2,00	0,080	-2,3	-5,9	a	--
L12	NRL/625	D	1,02	0,30	2,00	0,150	0,1	0,1	a	C2L
L13	NRL/625	D	0,93	0,28	2,00	0,140	-0,3	-0,5	a	NC
L14	OCL	NT								?
L15	NRL/625	D	1,13	0,17	2,00	0,085	0,5	1,3	a	NC
L16	NRL/625	D	0,96	0,28	2,00	0,140	-0,2	-0,3	a	NC
L17	OCL	D	0,89	0,26	2,00	0,130	-0,4	-0,8	a	NC
L18	OCL	NT								?
L19	NRL/120	D	1,03	0,26	2,00	0,130	0,1	0,2	a	NC
L20	NRL/625	D	1,05	0,16	2,00	0,080	0,2	0,5	a	NC
L21	NRL/625	D	1,29	0,52	2,00	0,260	1,2	1,1	a	NC
L22	NRL/625	D	1,03	0,27	2,00	0,135	0,1	0,2	a	NC
L23	NRL/625	D	1,02	0,20	2,00	0,100	0,1	0,2	a	NC
L24	NRL/120	D	1,04	0,10	2,00	0,050	0,2	0,5	b	NC
L25	NRL/625	D	0,64	0,24	2,00	0,120	-1,4	-2,7	a	NC
L26	NRL/625	D	0,96	0,27	2,00	0,135	-0,2	-0,3	a	NC
L27	NRL/625	D	0,78	0,05	1,73	0,029	-0,9	-3,5	b	NC
L28	NRL/625	D	1,26	0,23	2,00	0,115	1,0	2,0	a	NC
L29	NRL/625	D	0,89	0,20	2,00	0,100	-0,4	-1,0	a	NC
L30	NRL/625	D	1,00	0,16	4,30	0,037	0,0	0,0	b	NC
L31	NRL/625	D	1,57	0,37	1,73	0,214	2,3	2,6	a	NC
L32	NRL/625	NT								?
L33	NRL/625	D	0,94	0,33	2,00	0,165	-0,2	-0,3	a	C2L
L34	NRL/120	D	0,79	0,27	1,60	0,169	-0,8	-1,2	a	NC
L35	NRL/625	NT								?
L36	OCL	NT								?
L37	OCL	NT								--
L38	OCL	D	1,91			0,000	3,6	16,5	b	NC
L39	NRL/625	D	0,62	0,38	1,73	0,220	-1,5	-1,7	c	NC
L40	NRL/120	D	0,80	0,22	3,18	0,069	-0,8	-2,3	a	NC
L41	NRL/625	D	0,88	0,26	2,00	0,130	-0,5	-0,9	a	NC

Lab Code	Type	ID	x_i	±	k	$u(x_i)$	z score	ζ score	MU	Compl.
L42	NRL/625	D	1,01	0,20	2,07	0,097	0,0	0,1	a	NC
L43	NRL/120	D	1,04	0,08	2,57	0,031	0,2	0,6	b	C2L
L44	OCL	NT								?
L45	NRL/625	D	1,10	0,25	2,00	0,125	0,4	0,7	a	NC
L46	NRL/625	D	0,98	0,11	2,00	0,055	-0,1	-0,3	a	NC
L47	NRL/120	D	0,98	0,11	2,00	0,055	-0,1	-0,3	a	NC
L48	NRL/625	D	1,06	0,32	2,00	0,160	0,2	0,4	a	--
L49	NRL/625	D	0,98	0,29	2,00	0,147	-0,1	-0,1	a	NC
L50	NRL/120	D	0,99	0,11	1,73	0,064	0,0	-0,1	a	NC
L51	NRL/120	D	1,02	0,11	2,00	0,055	0,1	0,3	b	NC
L52	NRL/120	D	1,07	0,23	2,00	0,115	0,3	0,5	a	NC
L54	OCL	D	0,95	0,08	2,00	0,040	-0,2	-0,7	b	NC
L55	OCL	D	1,22	0,28	1,73	0,162	0,9	1,3	a	--
L56	OCL	NT								?
L57	NRL/625	D	0,97	0,04	2,00	0,020	-0,1	-0,5	b	C2L
L58	OCL	NT								--
L59	NRL/625	D	0,96	0,29	1,73	0,168	-0,2	-0,2	a	NC
L60	NRL/120	D	0,87	0,30	2,00	0,150	-0,5	-0,8	a	NC
L61	OCL	D	1,01	0,22	2,00	0,110	0,0	0,1	a	NC
L63	NRL/120	D	1,29	0,39	2,00	0,193	1,1	1,4	a	NC
L65	NRL/625	D	0,94	0,34	2,00	0,170	-0,2	-0,3	a	NC
L66	NRL/625	D	0,97	0,25	2,00	0,125	-0,1	-0,2	a	C2L
L67	OCL	NT								--
L68	NRL/625	D	1,02	0,19	2,00	0,095	0,1	0,2	a	NC
L69	NRL/625	D	1,25	0,63	2,00	0,315	1,0	0,8	c	--
L70	NRL/120	D	1,07	0,06	2,37	0,025	0,3	1,2	b	NC
L71	NRL/625	NT								?
L72	OCL	D	1,39	0,31	2,00	0,155	1,6	2,4	a	NC
L73	OCL	D	1,06			0,000	0,2	1,1	b	NC
L74	NRL/120	D	1,14	0,54	2,00	0,270	0,6	0,5	a	NC
L75	OCL	D	1,08	0,08	2,00	0,040	0,3	1,2	b	C2L
L76	OCL	D	0,96	0,14	2,00	0,070	-0,2	-0,4	a	C2L



Measurement result range reported by participants

Assigned value (x_{pt}): solid black line; Assigned range ($x_{pt} \pm U_{pt}$ (k=2)): dashed blue lines; Acceptance range ($x_{pt} \pm 2\,\sigma_{pt}$): dotted red lines.



Measurement result range reported by participants

Assigned value (x_{pt}): solid black line; Assigned range ($x_{pt} \pm U_{pt}$ (k=2)): dashed blue lines; Acceptance range ($x_{pt} \pm 2\,\sigma_{pt}$): dotted red lines.

Annex 6: Results of the questionnaire

T1: Please select the option that applies and proceed with the questionnaire (you may need to wait a few seconds before all additional questions open).

		Answers	Ratio
T1 was not analysed: go to Q1		2	3.03%
T1 was analysed: go to Q2		64	96.97%
No Answer		0	0%

T1: 1. Why did you not analyse test item 1?

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		2	3.03%
b) The methods are not validated in our laboratory		0	0%
c) We could not obtain sufficient good quality DNA suitable for further analysis		0	0%
d) Reference material, primers, probes, or other reagents were not available (in time)		0	0%
e) We tried but our analysis failed		0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)		0	0%
g) Other reason		0	0%
No Answer		64	96.97%

T1: 2. Select the DNA extraction method used for T1

		Answers	Ratio
CTAB		26	39.39%
NucleoSpin Food		17	25.76%
NucleoSpin Plant		3	4.55%
GeneSpin		4	6.06%
Promega Wizard		3	4.55%
DNeasy Plant		1	1.52%
DNeasy Mericon Food		2	3.03%
Biotecon Foodproof		3	4.55%
SDS		3	4.55%
Fast ID Genomic DNA		0	0%
CTAB + Maxwell 16 Food, Feed, Seed		5	7.58%
Generon Ion Force		2	3.03%
Other		3	4.55%
No Answer		2	3.03%

T1: 3. Select any additional DNA purification method used for T1.

		Answers	Ratio
No additional clean-up		47	71.21%
Additional ethanol precipitation		4	6.06%
Eurofins DNAExtractor cleaning column		4	6.06%
Promega Wizard DNA clean-up resin		3	4.55%
Qiagen QIAQuick		4	6.06%
Qiagen Genomic-Tip 20/G		0	0%
NucleoSpin gDNA clean-up		0	0%
Other method (no need to specify)		3	4.55%
No Answer		2	3.03%

T1: 4. What was the sample intake used for DNA extraction (in mg powder)?

		Answers	Ratio
500 mg or more		16	24.24%
300-400 mg		2	3.03%
250 mg		1	1.52%
200 mg		43	65.15%
150 mg		0	0%
100 mg		4	6.06%
<100 mg		0	0%
No Answer		2	3.03%

T1: 5a. What was the average DNA concentration (in ng/µL) in the final DNA extracts?

		Answers	Ratio
<10 ng/µL		1	1.52%
11-20 ng/µL		3	4.55%
21-30 ng/µL		6	9.09%
31-40 ng/µL		4	6.06%
41-50 ng/µL		3	4.55%
51-74 ng/µL		9	13.64%

75-100 ng/µL		9	13.64%
>100 ng/µL		26	39.39%
Not determined		5	7.58%
No Answer		2	3.03%

T1: 5b. How did you determine the DNA concentration indicated above?

		Answers	Ratio
Spectrophotometer (Nanodrop, Qubit, ...)		53	80.3%
Fluorometrically (Picogreen, Hoechst, ...)		6	9.09%
Quantitative PCR		0	0%
Other method		0	0%
Not determined		5	7.58%
No Answer		2	3.03%

T1: 6. Select the approach(es) used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		1	1.52%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		32	48.48%
We verify that the amplification curves look normal		28	42.42%
We run two dilutions and verify if the delta Cq or GM% are as expected		28	42.42%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		7	10.61%
We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq for undiluted extract, compare this to the measured Cq		14	21.21%
We add an internal positive control to the reactions and check the Cq		11	16.67%
Other		2	3.03%
No Answer		2	3.03%

T1: 7. Did you use a step-wise screening approach to find the GMO(s) present in the sample?

		Answers	Ratio
Yes, screening methods were applied first, followed by event-specific methods: continue with Q8		54	81.82%
No, GMO identification was immediately done with event-specific methods: go to Q10		10	15.15%
No Answer		2	3.03%

T1: 8. Select the screening methods applied to the sample and the outcome.

Screening Marker	Present	Absent	Unclear	No Answer
p35S	54	0	0	12
tNOS	54	0	0	12
PAT	0	36	4	26
BAR	0	33	4	29
CP4-EPSPS	13	1	5	47
Cry1Ab/Ac	0	17	4	45
Cry1Ab	0	1	6	59
pFMV	0	22	5	39
pNOS	0	2	7	57
t35S	0	3	7	56
nptII	0	12	5	49
p35S-pat	0	8	5	53
Ctp2-CP4-EPSPS	30	0	2	34
tE9	0	2	7	57
Other	5	4	2	55

T1: 9. Based on the screening results, which GM maize events could potentially be present in the sample?

Answers not shown

T1: 10. If applicable, why did you not test or quantify all GM events in T1?

		Answers	Ratio
a) Not applicable, all GM events listed were tested		33	50%
b) Based on the initial screening tests we could reduce the number of event-specific tests to perform		21	31.82%
c) The event-specific detection method is not validated in our laboratory		6	9.09%
d) Reference material, primers, probes, or other reagents were not		7	10.61%

available (in time)			
e) The result obtained was below the LOD/LOQ		1	1.52%
f) Practical constraints (instrument broken, no personnel, etc.)		1	1.52%
g) Other reason		0	0%
No Answer		2	3.03%

T1: 11. Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		51	77.27%
Delta Cq method (one calibration curve)		6	9.09%
Digital PCR (no calibration curve)		1	1.52%
No quantification done: go to Q20		7	10.61%
No Answer		2	3.03%

T1: 12. Select the calibrant used for the standard curve.

		Answers	Ratio
CRM from JRC (ex-IRMM), certified in GM mass fraction (g/kg)		55	83.33%
CRM from AOCS, certified for purity (assumed 1000 g/kg)		1	1.52%
CRM from JRC (ex-IRMM), certified in number of DNA fragments per plasmid (indicative GM copy number ratio)		1	1.52%
Non-certified RM (*e.g.* lab QC material), expressed in GM mass fraction (g/kg or m/m %)		0	0%
Non-certified RM (*e.g.* lab QC material), expressed in GM DNA copy number ratio (*e.g.* determined by digital PCR)		0	0%
No calibrant used, digital PCR done		0	0%
No Answer		9	13.64%

T1: 13. Select the endogenous target(s) used for relative quantification.

		Answers	Ratio
adh1 - 70 bp		14	21.21%
adh1 - 134-136 bp		9	13.64%
Hmg		33	50%
zSSIIb		0	0%
Invertase		1	1.52%
Other, please specify below		0	0%
No Answer		9	13.64%

T1: 14. Clarify the unit of measurement used and any conversion between units if applicable. Carefully read the choices below and select the one used in the measurements that resulted in a final result in GM m/m %. If unclear or a different approach was used, please clarify this in the free text box below.

		Answers	Ratio
The RM and the calibration standards were expressed in mass (or mass %), no conversion factor was applied		39	59.09%
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, and no conversion factor was applied (*e.g.* 10 % m/m GM = 10 % cp/cp GM)		11	16.67%
The calibration standards were expressed in DNA copies, calculated from the RM in g/kg, and a conversion factor >1 was applied to take account of the zygosity and target gene copies; a conversion factor (*e.g.* 2) was used to convert from mass to copies (*e.g.* 10 % m/m GM = 5 % cp/cp GM); the final result was again converted to m/m % by using the same conversion factor (double conversion applied). Please specify this factor below.		3	4.55%
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). A conversion factor (different from 1) was applied onto the final GM %, please specify this factor below.		3	4.55%
The measurements were done in DNA copies (as the RM used was expressed in this unit or digital PCR was used). No conversion factor was applied onto the final GM %.		1	1.52%
Other		0	0%
No Answer		9	13.64%

Conversion factor used to turn results into m/m %, if applicable, and/or clarification on preparation of standards.

L21	1.67
L35	0.56
L57	2
L61	2

T1: 15. What was the amount of sample DNA (ng) used per PCR. Choose the amount that is closest to what you used. If applicable, select multiple choices (*e.g.* if several dilutions were tested) but only those of which the result was used to determine the reported GM %.

		Answers	Ratio
DNA concentration not determined		5	7.58%
250 ng		3	4.55%
200 ng		21	31.82%
150 ng		5	7.58%
100 ng		19	28.79%
50 ng		10	15.15%
25 ng		6	9.09%
15 ng		4	6.06%
<10 ng		1	1.52%
No Answer		9	13.64%

T1: 16. Enter the slope of the calibration curve for the GM gene

Lower than -3.6	10
Between -3.1 and -3.6	42
Higher than -3.10	3

T1: 17. Enter the slope of the calibration curve for the taxon-specific reference gene

Lower than -3.6	2
Between -3.1 and -3.6	51
Higher than -3.10	2

T1: 18. Were the Cq values obtained for the sample all within the calibration curve range?

		Answers	Ratio
Yes		52	78.79%
No		5	7.58%
No Answer		9	13.64%

T1: 19. How did you estimate the measurement uncertainty on the result reported?

		Answers	Ratio
Uncertainty budget (ISO GUM)		4	6.06%
Uncertainty of the method (in-house validation)		16	24.24%
Known uncertainty of the standard method		5	7.58%
Measurement of replicates (precision)		26	39.39%
From interlaboratory comparison data		2	3.03%
Estimation based on judgement		1	1.52%
In another way, please specify below		5	7.58%
No Answer		9	13.64%

T1: 20. Based on your measurement results do you consider the sample compliant with the EU GMO legislation? Select the option that applies.

		Answers	Ratio
Compliant and no labelling required (if GMO presence is adventitious or technically unavoidable)		3	4.55%
Compliant if labelled as containing GMO		48	72.73%
Not compliant		5	7.58%
Cannot be concluded		8	12.12%
No Answer		2	3.03%

T1: 21. Justify the compliance statement provided.

		Answers	Ratio
1. GM event is authorised in EU and GM mass fraction measured is equal to or lower than 0.9 % for all GM events per species		3	4.55%
2. GM event is authorised in EU and GM mass fraction is higher than 0.9 % for all GM events per species		50	75.76%
3. GM event is not authorised in the EU		0	0%
4. GM event falls under Regulation (EU) 619/2011 and mass fraction (+U) is equal to or below 0.1 %		0	0%
5. GM event falls under Regulation (EU) 619/2011 and mass fraction (+U) is higher than 0.1 %		2	3.03%
6. No quantification done (for all GM events identified), cannot conclude		7	10.61%
7. Other explanation		2	3.03%
No Answer		2	3.03%

T2: Please select the option that applies and proceed with the questionnaire (you may need to wait a few seconds before all additional questions open).

		Answers	Ratio
T2 was not analysed: go to Q1		2	3.03%
T2 was analysed: go to Q2		64	96.97%
No Answer		0	0%

T2: 1. Why did you not analyse test item 2?

		Answers	Ratio
a) The sample matrix is out of the scope of our laboratory		0	0%
b) The methods are not validated in our laboratory		0	0%
c) We could not obtain sufficient good quality DNA suitable for further analysis		0	0%
d) Reference material, primers, probes, or other reagents were not available (in time)		2	3.03%
e) We tried but our analysis failed		0	0%
f) Other practical constraints (instrument broken, no personnel, etc.)		0	0%
g) Other reason		0	0%
No Answer		64	96.97%

T2: 2. Select the DNA extraction method used for T2.

		Answers	Ratio
CTAB		28	42.42%
NucleoSpin Food		13	19.7%
NucleoSpin Plant		3	4.55%
GeneSpin		4	6.06%
Promega Wizard		4	6.06%
DNeasy Plant		0	0%
DNeasy Mericon Food		1	1.52%
Biotecon Foodproof		3	4.55%
SDS		3	4.55%
Fast ID Genomic DNA		0	0%
CTAB + Maxwell 16 Food, Feed, Seed		5	7.58%
Generon Ion Force		2	3.03%
Other		3	4.55%
No Answer		2	3.03%

T2: 3. Select any additional DNA purification method used for T2.

		Answers	Ratio
No additional clean-up		44	66.67%
Additional ethanol precipitation		4	6.06%
Eurofins DNAExtractor cleaning column		3	4.55%
Promega Wizard DNA clean-up resin		5	7.58%
Qiagen QIAQuick		6	9.09%
Qiagen Genomic-Tip 20/G		0	0%
NucleoSpin gDNA clean-up		0	0%
Other method (no need to specify)		3	4.55%
No Answer		2	3.03%

T2: 4. What was the sample intake used for DNA extraction (in mg powder)?

		Answers	Ratio
500 mg or more		5	7.58%
300-400 mg		3	4.55%
250 mg		4	6.06%
200 mg		47	71.21%
150 mg		2	3.03%
100 mg		4	6.06%
<100 mg		0	0%
No Answer		2	3.03%

T2: 5a. What was the average DNA concentration (in ng/µL) in the final DNA extracts?

		Answers	Ratio
<10 ng/µL		0	0%
11-20 ng/µL		0	0%
21-30 ng/µL		1	1.52%
31-40 ng/µL		1	1.52%
41-50 ng/µL		1	1.52%
51-74 ng/µL		4	6.06%
75-100 ng/µL		2	3.03%
>100 ng/µL		51	77.27%

Not determined		4	6.06%
No Answer		2	3.03%

T2: 5b. How did you determine the DNA concentration indicated above?

		Answers	Ratio
Spectrophotometer (Nanodrop, Qubit, ...)		52	78.79%
Fluorometrically (Picogreen, Hoechst, ...)		8	12.12%
Quantitative PCR		0	0%
Other method		0	0%
Not determined		4	6.06%
No Answer		2	3.03%

T2: 6. Select the approach(es) used to show absence of PCR inhibition.

		Answers	Ratio
None (no inhibition was suspected based on experience)		4	6.06%
We run two dilutions and verify if the delta Cq or GM% are as expected		28	42.42%
We run three or four dilutions and verify if the delta Cq or GM% are as expected		9	13.64%
We perform a PCR inhibition run with a reference gene before analysis: 3 or 4 dilutions, linear regression, extrapolation of Cq of undiluted extract, compare this to the measured Cq		14	21.21%
We add an internal positive control to the reactions and check the Cq		10	15.15%
We verify that the amplification curves look normal		22	33.33%
We check that the optical density ratios (OD260/280, 260/230) are acceptable		30	45.45%
Other		2	3.03%
No Answer		2	3.03%

T2: 7. If applicable, why did you not test or quantify all GM events in T2?

		Answers	Ratio
a) Not applicable, all GM events listed were tested		52	78.79%
b) Based on the initial screening tests we could reduce the number of event-specific tests to perform		3	4.55%
c) The event-specific detection method is not validated in our laboratory		4	6.06%
d) Reference material, primers, probes, or other reagents were not available (in time)		5	7.58%
e) The result obtained was below the LOD/LOQ		0	0%
f) Practical constraints (instrument broken, no personnel, etc.)		1	1.52%
g) Other reason		2	3.03%
No Answer		2	3.03%

T2: 8. Which quantification approach was used?

		Answers	Ratio
Standard curve method (2 calibration curves)		49	74.24%
Delta Cq method (one calibration curve)		6	9.09%
Digital PCR (no calibration curve)		2	3.03%
No quantification done, go to Q11		8	12.12%
No Answer		2	3.03%

T2: 9. Select the calibrant used for the standard curve.

		Answers	Ratio
CRM from JRC (ex-IRMM), certified in GM mass fraction (g/kg)		55	83.33%
CRM from AOCS, certified for purity (assumed 1000 g/kg)		1	1.52%
CRM from JRC (ex-IRMM), certified in number of DNA fragments per plasmid (indicative GM copy number ratio)		0	0%
Non-certified RM (*e.g.* lab QC material), expressed in GM mass fraction (g/kg or m/m %)		0	0%
Non-certified RM (*e.g.* lab QC material), expressed in GM DNA copy number ratio (*e.g.* determined by digital PCR)		0	0%
No calibrant used, digital PCR done		0	0%
No Answer		10	15.15%

T2: 10. Select the endogenous target(s) used for relative quantification.

		Answers	Ratio
adh1 - 70 bp		0	0%
adh1 - 134-136 bp		6	9.09%
Hmg		48	72.73%
zSSIIb		0	0%
Invertase		1	1.52%

Other, please specify below		1	1.52%
No Answer		10	15.15%

T2: 11. Based on your measurement results do you consider the sample compliant with the EU GMO legislation? Select the option that applies.

		Answers	Ratio
Compliant and no labelling required (if GM presence is adventitious or technically unavoidable)		1	1.52%
Compliant if labelled as containing GMO		7	10.61%
Not compliant		47	71.21%
Cannot say, no quantification done		9	13.64%
No Answer		2	3.03%

T2: 12. Justify the compliance statement provided.

		Answers	Ratio
1. GM event is authorised in EU and GM mass fraction measured is equal to or lower than 0.9 % for all GM events per species		2	3.03%
2. GM event is authorised in EU and GM mass fraction is higher than 0.9 % for all GM events per species		5	7.58%
3. GM event is not authorised in the EU		13	19.7%
4. GM event falls under Regulation (EU) 619/2011 and mass fraction (+U) is equal to or below 0.1 %		0	0%
5. GM event falls under Regulation (EU) 619/2011 and mass fraction (+U) is higher than 0.1 %		35	53.03%
6. No quantification done (for all GM events identified), cannot conclude		8	12.12%
7. Other explanation		1	1.52%
No Answer		2	3.03%

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