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JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS

Comparative Testing Report on the Detection and Quantification of Soybean Event 40-3-2

Comparative testing round: ILC-EURL-GMFF-CT-01/11

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Molecular Biology and Genomics Unit

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Executive Summary

The Joint Research Centre (JRC) as European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF), established by Regulation (EC) No 1829/2003[(1)], organised a comparative testing round for National Reference Laboratories (NRLs) nominated under Regulation (EC) No 882/2004[(2)] and Regulation (EC) No 1981/2006[(3)], for Official control laboratories and for laboratories from third countries which had volunteered to participate.

In accordance with Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the EU-RL GMFF shall organise comparative testing and shall ensure an appropriate follow-up of such testing.

The design and execution of the comparative testing round was in accordance with the ISO 17043 Standard[(4)]. The EU-RL GMFF is accredited according to the ISO 17043 Standard 'General requirements for proficiency testing'[(4)].

The test items used in the comparative testing round ILC-EURL-GMFF-CT-01/11 were produced in-house. Monsanto provided soybean seeds containing the transformation event. Participants had to determine the genetically modified (GM) content in two test items denoted soybean powder levels 1 and 2, containing different GM percentages of soybean event 40-3-2 flour (unique identifier MON-Ø4Ø32-6). In February 2011, a total of 155 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/11. Eight NRLs declined participation, of which one was no longer a NRL. One hundred and nine laboratories registered for this comparative testing round. Test items were shipped to the participants at the beginning of April 2011 in plastic containers containing approximately 5 g of flour. One hundred and two laboratories from 43 countries returned results, which fell into the following groups:

1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1)
2. 28 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
3. 31 were NRLs nominated under both Regulations (group 3),
4. 11 were only members of the European Network of GMO Laboratories (ENGL, group 4),
5. 8 were only Official control laboratories (group 5),
6. 21 were laboratories from third countries (group 6).

Five NRLs (group 3) submitted results in both measurement units. Seven laboratories including one NRL (group 3), one ENGL member (group 4) and five laboratories from a third country (group 6) did not submit results. The Food Safety and Quality (FSQ) Unit of the Institute for Reference Materials and Measurements (IRMM) managed the on-line registration and submission of results.

Participants could report the results in either mass/mass % (m/m %) or copy/copy % (cp/cp %). The EU-RL GMFF calculated the robust means (μ_R) of the soybean powder levels

1 and 2 test items in m/m % and in cp/cp %. All data were log-transformed and then robust statistics were applied to obtain a robust mean [5, 6, 7]. The homogeneity and stability studies were conducted at the EU-RL GMFF. These data were included in the uncertainty budget.

The target standard deviation for comparative testing $\hat{\sigma}$ for soybean event 40-3-2 was fixed at 0.15 ($\log_{10}$ value) by the Advisory Board for Comparative testing. This target standard deviation was used to derive z-scores for the participants' results. An overview of the robust means and number of z-scores in the range of -2 to +2 is given in Figure 1.

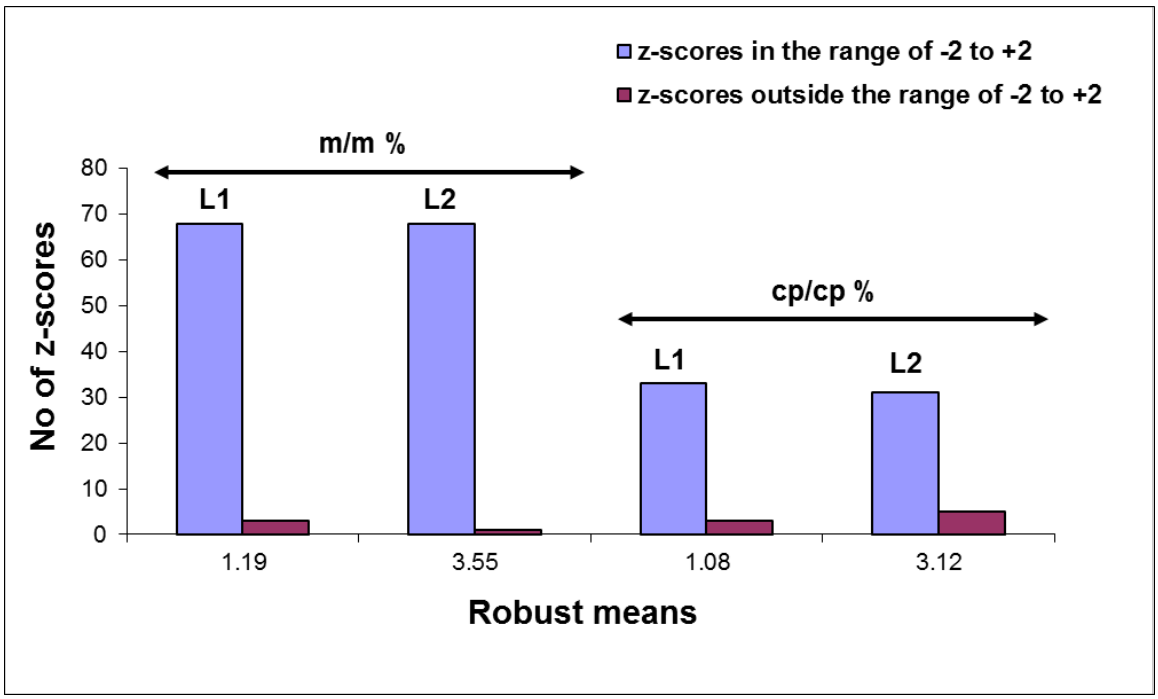

Figure 1. Overview of z-scores calculated on the basis of robust means. m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, L1 = level 1, L2 = level 2.

In this third comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for both soybean powder levels 1 and 2 regardless of the calibration method and the measurement unit.

Participants' assessment of results in relation to measurement uncertainty (MU) needs to be improved because only about 56 % of participants provided information on MU in a complete and consistent manner.

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1. Introduction

The Joint Research Centre (JRC) as European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF) was established by Regulation (EC) No 1829/2003[1]. The EU-RL GMFF has two mandates determined by Regulation (EC) No 1981/2006[3] and by Regulation (EC) No 882/2004[2].

In accordance with Article 32 of Regulation (EC) No 882/2004 the EU-RL GMFF shall organise comparative testing for National Reference Laboratories (NRLs) and shall ensure an appropriate follow-up of such testing. The aim of this activity is 'to contribute to a high quality and uniformity of analytical results'[2]. Moreover, Article 12 of Regulation (EC) No 882/2004 states that the nominated NRLs should be accredited in accordance with ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. One of the requirements of ISO/IEC 17025 accredited laboratories is to prove their competence by taking part in a proficiency testing scheme.

Regulation (EC) No 1829/2003 establishes a threshold for labelling of food and feed products consisting of or containing more than 0.9 % genetically modified organisms (GMOs) provided the GMO has undergone the authorisation procedure in accordance with European Union legislation. This threshold is used by the Member States of the European Union involved in the official control of food and feed. Hence, an accurate determination of the GM content in sampled products is of paramount importance.

In 2011 the EU-RL GMFF organised the third comparative testing round in collaboration with the Food Safety and Quality (FSQ) Unit of the Institute for Reference Materials and Measurements (IRMM). The comparative testing round was announced at the European Network of GMO Laboratories (ENGL) plenary meeting on the 9th and 10th of November 2010. In February 2011, a total of 155 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/11. Eight NRLs declined participation, of which one was no longer a NRL. One hundred and nine laboratories registered for this comparative testing round. Test items were shipped between the 4th and 6th of April 2011. The deadline for submission of results was the 20th of May 2011. The FSQ Unit of IRMM managed the on-line registration and submission of results employing a database of the International Measurement Evaluation Programme (IMEP).
One hundred and two laboratories from 43 countries returned results, which fell into the following groups:

1. 3 were NRLs nominated only under Regulation (EC) No 882/2004 (group 1)
2. 28 were NRLs nominated only under Regulation (EC) No 1981/2006 (group 2),
3. 31 were NRLs nominated under both Regulations (group 3),
4. 11 were only ENGL members (group 4),
5. 8 were only Official control laboratories (group 5),
6. 21 were laboratories from third countries (group 6).

Five NRLs (group 3) submitted results in both measurement units. Seven laboratories including one NRL (group 3), one ENGL member (group 4) and five laboratories from a third

country (group 6) did not submit results. The FSQ Unit of IRMM managed the on-line registration and submission of results.

2. Description of the comparative test items

2.1 Preparation

Test items were prepared in-house in accordance with ISO Guide 34[8] regarding the 'General requirements for the competence of reference material producers'.

Soybean powder levels 1 and 2 were prepared to nominal values of 0.90 m/m % and 2.80 m/m % GM of 40-3-2 flours, respectively.

The preparation of test items was carried out from the end of October 2010 until the beginning of May 2011. Raw materials (seeds) were assessed for basic seed traits (i.e. water content) and for the presence of other GM events authorised within the European Union. The zygosity of the event 40-3-2 was assessed in the GM line. Powders of non-modified and event 40-3-2 soybean were prepared by a one-step grinding process using an Ultra Centrifugal Mill ZM200 (Retsch GmbH, DE) and tested for DNA extractability using the Macherey-Nagel (Düren, DE) plant DNA extraction kit, and a validated CTAB DNA extraction method. Test items were obtained in a one-step dilution by mixing non-modified soybean powder and 40-3-2 soybean powder in specified mass proportions corrected for the water content.

Approximately 5 g of the dry-mixed test items were aliquoted in 30-mL plastic tubs using an automatic sampling device, and labelled as soybean powder levels 1 or 2. Test items were stored at +4 °C in the dark.

2.2 Homogeneity and stability assessment

The assessment of the homogeneity[9] was performed after the test items had been packed in their final form and before distribution to participants.

Samples are considered to be adequately homogeneous if:

$$s_s \leq 0.3\hat{\sigma} \qquad (1)$$

Where s_s is the between-test item standard deviation as determined by a single factor ANOVA[10] and $\hat{\sigma}$ is the standard deviation for comparative testing.

If this criterion is met, the between-test item standard deviation contributes no more than about 10 % to the standard deviation for comparative testing.

The repeatability of the test method is the square root of mean sum of squares within-test item MS_{within}. The relative between-test item standard deviation $s_{s,rel}$ is given by

$$s_{s,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \times 100\% \quad (2)$$

where: $MS_{between}$ is the mean sum of squares between test items
MS_{within} is the mean sum of squares within test items
n is the number of replicates
$\bar{y}$ is the mean of the homogeneity data

If $MS_{within} > MS_{between}$ then:

$$s_{s,rel} = u^*_{bb} = \frac{\frac{repeatability}{\sqrt{n}} \sqrt[4]{\frac{2}{N(n-1)}}}{\bar{y}} \times 100\% \quad (3)$$

where u^*_{bb} is the maximum uncertainty contribution that can be obtained by the hidden heterogeneity of the material.

For each GM level ten test items (N = 10) were randomly selected and analysed in five-fold replicates (n = 5). The criterion described in formula (1) was fulfilled thus indicating that both soybean powder test items were homogeneous.

The data from the homogeneity study conducted at the EU-RL GMFF were used for the estimation of the uncertainty contributions related to the homogeneity and to the stability of the soybean powder levels 1 and 2 test items, respectively.

An isochronous short term stability study involving two soybean powder level 1 test items (N = 2, n = 3) was conducted for time periods of one, two and four weeks at temperatures of +4 ºC, +18 ºC and +60 ºC [11]. The results of the study did not reveal any influence of the temperature on the stability of test items, and consequently they could be shipped to participants at ambient temperature.

An isochronous long term stability study involving two soybean powder level 1 test items (N = 2, n = 3) was conducted for time periods of five, nine and twelve months at a temperature of +4 ºC[11]. No significant trend (95 % confidence level) was detected thus indicating that test items can be stored at +4 ºC.

3. Participants' results

The assignment of a laboratory number to each participant and the submission of results were managed by the FSQ Unit of IRMM. Results had to be reported on-line for which each participant received an individual access code. A questionnaire was attached to the on-line reporting form to collect details of the analytical methods used.

Participants could report the results of the exercise in either m/m % or cp/cp %. The expression of measurement results in cp/cp % follows the Recommendation (EC) No 2004/787[12], where it is recommended that the results of quantitative analyses are expressed as GM DNA copy numbers in relation to target taxon-specific copy numbers calculated in terms of haploid genomes.

Participants were instructed to apply the formulas described below when reporting their results.

$$\text{m/m \%} = \frac{\text{mass GM event [g]}}{\text{Total soybean mass [g]}} \times 100\ \% \qquad (4)$$

$$\text{cp/cp \%} = \frac{\text{GM event DNA copy numbers [cp]}}{\text{Target taxon-specific DNA copy numbers [cp]}} \times 100\ \% \qquad (5)$$

A total of 102 laboratories from 43 countries reported results (Figures 2 and 3). Seventy-one laboratories reported the GM content in m/m % (Figure 4). One laboratory submitted two sets of results in m/m %. Thirty-six laboratories expressed their results in cp/cp % (Figure 4) of which 30 used a genomic and 6 laboratories used a plasmid DNA calibrant. Five laboratories reported the results in both measurement units (Figure 4). Seven laboratories including one NRL, one ENGL only member and five laboratories from third countries did not submit any results.

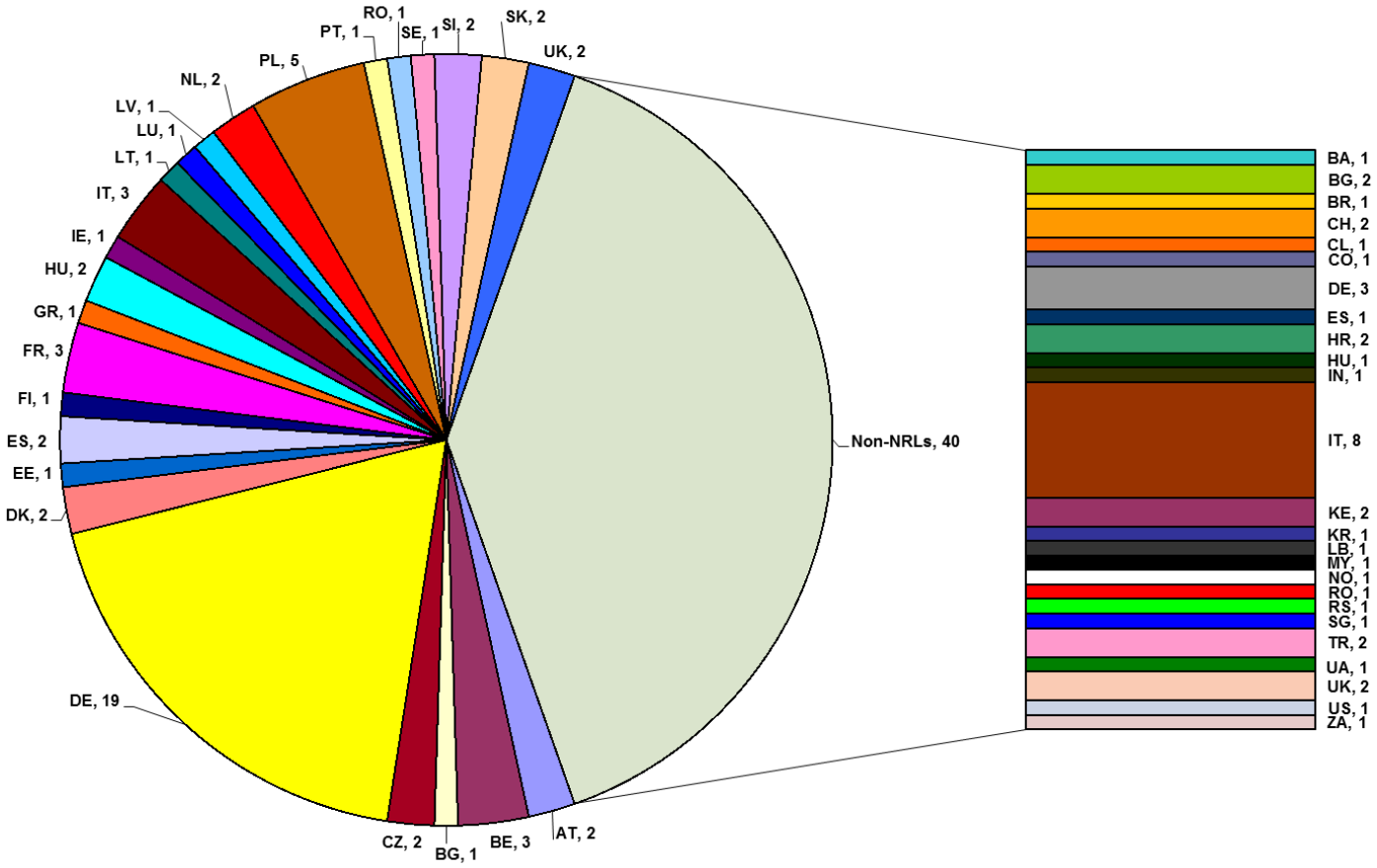

Figure 2: Distribution of participants from different countries

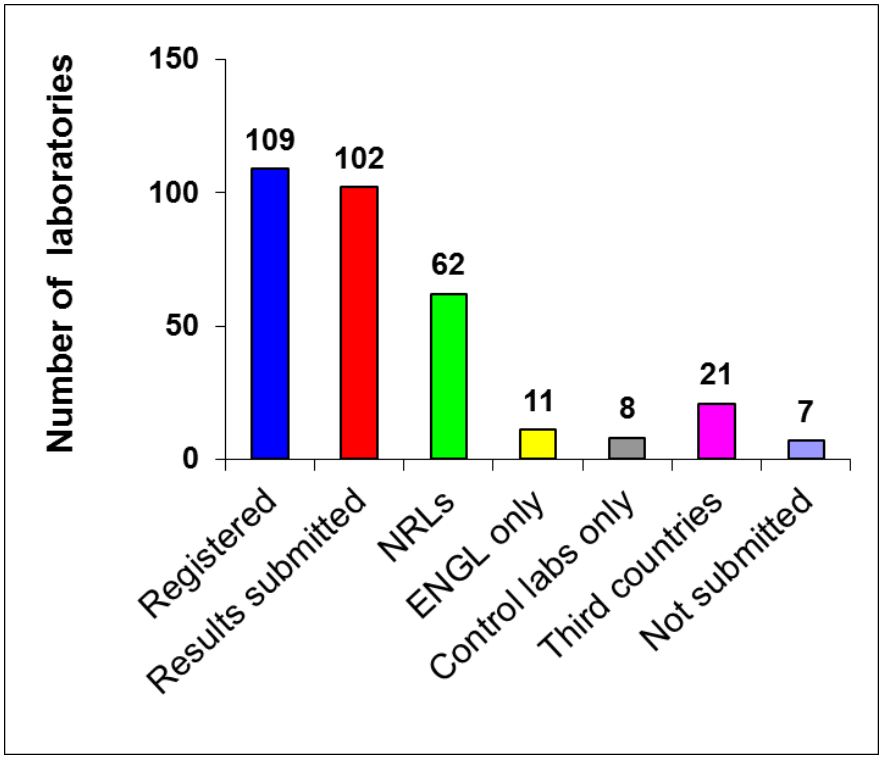

Figure 3. Overview of participants' results grouped by type of laboratory.

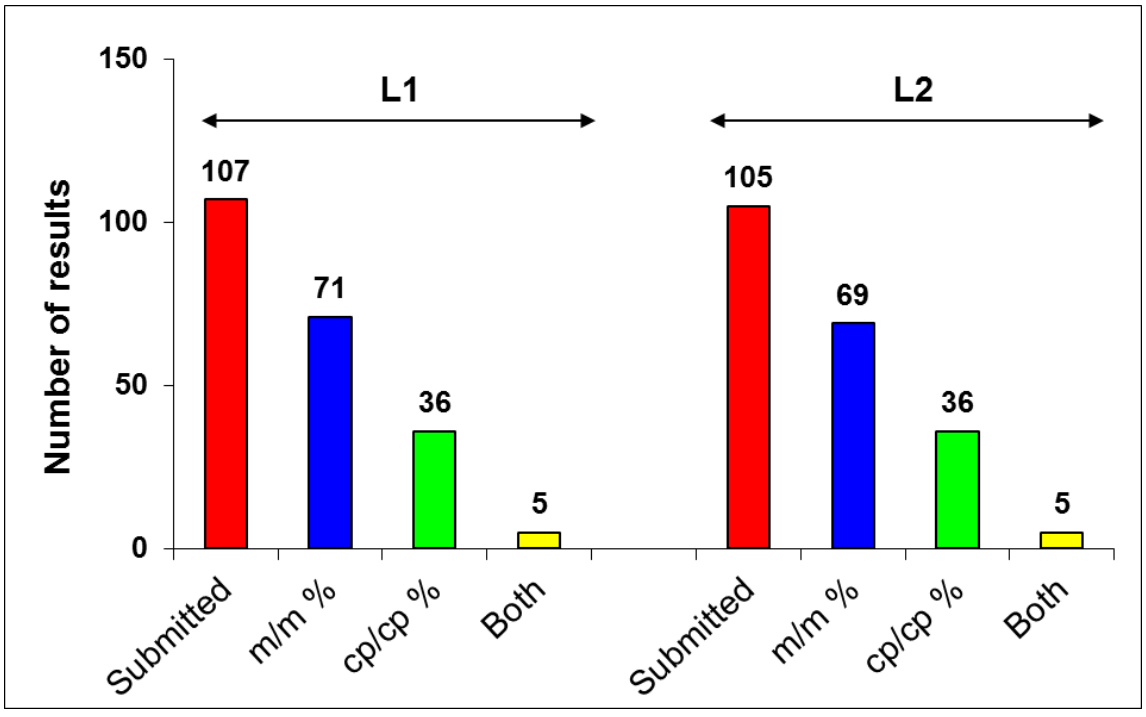

Figure 4. Overview of participants' results grouped by GM level and measurement unit. m/m % = results submitted in m/m %, cp/cp % = results submitted in cp/cp %, Both = results submitted in both measurement units, L1 = level 1, L2 = level 2.

The EU-RL GMFF calculated the robust means (μ_R) of the soybean powder levels 1 and 2 test items in m/m % and cp/cp %. All data were log-transformed and then robust statistics

were applied to obtain a robust mean[5, 6, 7]. Data from the homogeneity and stability studies conducted by the EU-RL GMFF were included in the uncertainty budget.

An overview of the results reported in m/m % and cp/cp % is given in Tables 2 to 5. An overview of the analytical methods used by each participant is summarised in the section on 'Questionnaire data'.

4. Assigned value and measurement uncertainty

4.1 Consensus value from participants

The consensus value (μ_R) from participants in the comparative testing round was calculated using robust statistics[13]. This approach minimises the influence of outlying values. All results were log-transformed prior to the calculation of the robust mean to establish a near-normal distribution allowing the interpretation of results on the basis of a normal distribution[6]. Robust means (μ_R) were calculated on the basis of the results reported in m/m % and cp/cp %, respectively.

The expanded uncertainty (U) comprises standard uncertainty contributions from the characterisation of the material (u_{char}), the between-test item homogeneity (u_{bb}) and the long-term stability of the material (u_{lts})[14]. The uncertainty contribution from the characterisation of the material is calculated using formula (7). A coverage factor of 2 was used to calculate the expanded uncertainty corresponding to a 95 % level of confidence[15].

$$U = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2} \qquad (6)$$

The standard uncertainty (u_{char}) of the characterisation is calculated using the formula:

$$u_{char} = \frac{\sigma}{\sqrt{N}} \qquad (7)$$

where: σ = relative standard deviation of the robust mean
N = number of data points.

The consensus values of soybean powder levels 1 and 2 are traceable to the measurement unit of the reference material that was used for the preparation of the standard curves.

The robust means (μ_R) determined by the EU-RL GMFF are depicted in Table 1.

Table 1. Overview of robust means (μ_R) and expanded uncertainties for soybean powder levels 1 and 2

40-3-2 soybean content		**Relative standard uncertainty contributions**			**Expanded uncertainty (U = 2 * u_c)**	
μ_R **[m/m %]**		$(u_{char,rel})^1$	$(u_{bb,rel})^2$	$(u_{lts,rel})^3$	$U_{rel\,[\%]}$	$U_{abs\,[m/m\,\%]}$
Soybean powder level 1	1.18 (N = 71)	2.33	2.84	1.58	8.00	0.09
Soybean powder level 2	3.52 (N = 69)	2.05	2.52	1.58	7.23	0.25
μ_R **[cp/cp %]**					$U_{rel\,[\%]}$	$U_{abs\,[cp/cp\,\%]}$
Soybean powder level 1	1.05 (N = 36)	5.90	2.84	1.58	13.47	0.14
Soybean powder level 2	3.07 (N = 36)	4.34	2.52	1.58	10.52	0.32

[1] Relative standard uncertainty relating to the characterisation
[2] Standard uncertainty contribution resulting from the homogeneity assessment
[3] Relative standard uncertainty relating to the long-term stability, estimated on the basis of a shelf life of 12 months.

The standard uncertainty (u_{char}) of the characterisation tends to increase when the robust mean is calculated on the basis of a lower number of data points (Formula 7).

5. Statistical data and summaries

The aim of a performance statistic is to provide participants with a meaningful result that can be easily interpreted. The procedure followed for the evaluation of participants' performance was agreed by the Members of the Advisory Board and relies on the calculation of z-scores on the basis of the robust means (μ_R) of the participants' results[(9)]. Laboratories are compared on the basis of z-scores calculated from log-transformed data[(6)]. Participants reported results in m/m % and in cp/cp %. All results reported in cp/cp % were pooled irrespective of the DNA calibrant used (i.e. plasmid or genomic DNA) due to the limited number of results obtained with a plasmid DNA calibrant (N = 6).

The value of $\hat{\sigma}$, the target standard deviation for comparative testing, determines the performance limits in a comparative test and is set at a value that reflects best practice for the analysis in question. For this round the Members of the Advisory Board chose a value of 0.15[(16)]. The z-score (z_i) for participant i reporting measurement result x_i is thus calculated as

$$z_i = \left(\log_{10} x_i - \log_{10} \mu_R\right) \Big/ \hat{\sigma} \qquad (8)$$

where: μ_R = robust mean expressed in m/m % or cp/cp %

Table 2. z-scores for event 40-3-2 soybean powder level 1 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, z-score calculated on the basis of the robust mean, * no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (e) *U* seems to be underestimated, (f) *U* seems to be overestimated, (g) LOD/LOQ was reported in an inconsistent way, (h) LOD/LOQ seems to be reported in absolute copy numbers, (i) LOD/LOQ seem to be overestimated. Results are as submitted by participants.

Soybean event 40-3-2

Laboratory number	Robust mean = 1.18 m/m %					
	Value	Uncertainty relative	Uncertainty absolute	LOD m/m	LOQ m/m	z-score
L002	**0.97**		(b) 0.74	0.01	0.10	**-0.57**
L005	**1.12**		(a) 0.31	0.025	0.048	**-0.16**
L006	**0.72**	(b) (c) (e) 0.05		0.02	0.06	**-1.44**
L007	**1.27**		0.69	0.015	0.017	**0.21**
L009	**1.24**	30.00		<0.1	<0.1	**0.14**
L011	**1.50**	(a) 8.73		0.05	0.10	**0.69**
L013	**1.07**	(a) (c) 0.20		(g) 0.01	(g) 0.10	**-0.29**
L014	**1.12**		0.71	0.04	0.16	**-0.16**
L015	**1.42**	(a) 0.83		0.10	0.10	**0.53**
L016	**1.39**		(a) 0.22	0.02	0.10	**0.47**
L017	**1.18**		0.36	0.01	0.03	**-0.01**
L019	**1.12**	(a) (c) 0.34		0.04	-	**-0.16**
L020	**1.20**	(a) (c) 0.02		0.045	0.10	**0.04**
L021	**1.00**		0.11	0.045	0.09	**-0.49**
L022	**0.93**		0.32	-	-	**-0.70**
L023	**1.30**	(a) 15.00		-	-	**0.27**
L024	**1.18**	(c) 0.20		0.05	0.10	**-0.01**
L027	**1.13**		(e) 0.06	0.05	0.10	**-0.13**
L030	**0.90**	(c) 0.30		0.001	0.03	**-0.79**
L031	**1.22**		0.42	0.03	0.09	**0.09**
L032	**1.27**		(a) 0.42	0.50	0.50	**0.21**
L033	**0.99**		0.66	0.01	0.12	**-0.52**
L034	**0.26**		(e) 0.08	0.01	0.1	**-4.39**
L036	**1.36**		0.45	0.026	-	**0.40**
L037	**1.04**	(b) (c) 0.28		0.05	0.1	**-0.37**
L038	**0.80**	(c) 0.20		0.003	0.04	**-1.13**
L039	**1.13**		(b) 0.23	0.02	0.10	**-0.13**
L041	**0.78**	-	-	(g) 0.10	(g) 0.10	**-1.21**
L042	**1.01**		0.10	0.05	0.10	**-0.46**
L043	**1.13**		0.34	0.01	0.04	**-0.13**
L044	**1.01**	(a) (c) 0.12		-	-	**-0.46**
L045	**1.36**		(a) 0.24	-	0.10	**0.40**
L046	**1.36**		0.50	0.01	0.10	**0.40**
L047	**1.78**		(a) 0.30	0.04	0.08	**1.18**
L048	**1.26**	(a) 40.00		<0.1	0.10	**0.18**
L050	**1.19**	(c) 0.11		0.1	0.10	**0.02**
L051	**1.21**		(a) (e) 0.05	-	-	**0.07**
L053	**0.99**	(c) 0.50		0.05	0.10	**-0.52**
L054	**1.78**		0.50	0.10	0.10	**1.18**
L055	**1.40**		0.40	0.04	0.10	**0.49**
L056	**0.93**	(c) 0.29		(g) -1.00	(g) -1.00	**-0.70**
L059	**1.08**		0.11	0.10	0.40	**-0.26**
L061	**1.41**		0.38	0.01	0.10	**0.51**
L062	**2.10**		0.32	0.045	0.09	**1.66**
L063	**1.15**		0.27	0.03	0.08	**-0.08**
L064	**1.33**		(e) 0.06	0.02	0.06	**0.34**
L065	**1.45**		(d) (e) 4.79	0.045	0.10	**0.59**
L067	**0.95**	-	-	0.045	0.09	**-0.64**
L068	**1.22**		(a) 0.77	0.05	0.05	**0.09**
L072	**1.11**	(a) 32.98		0.10	0.10	**-0.18**
L074	**1.07**		(a) 0.07	0.10	0.29	**-0.29**
L075	**< 1.00**	-	-	<1.00	1.00	*****
L076	**1.09**	-	-	0.10	-	**-0.24**
L078	**0.93**		0.67	0.0019	0.016	**-0.71**
L079	**1.11**	(a) (c) 0.27		0.04	0.13	**-0.20**
L080	**1.15**	(c) 0.73		0.01	0.06	**-0.08**
L082	**4.70**		(a) 0.30	0.10	0.10	**3.99**
L084	**1.60**		(a) 0.66	0.02	0.10	**0.87**
L086	**1.70**		(a) 0.30	0.05	0.10	**1.05**
L089	**1.10**		(e) 0.09	0.02	0.10	**-0.21**
L092	**0.98**	-	-	0.007	0.013	**-0.55**
L093	**1.32**	(b) (c) 0.20		0.01	0.10	**0.32**
L095	**1.45**	-	-	0.01	0.10	**0.59**
L097	**1.29**		0.46	(i) 0.51	(i) 1.15	**0.25**
L098	**1.56**	(c) 0.14		0.045	0.10	**0.80**
L101	**1.11**		(e) 0.07	0.03	0.10	**-0.18**
L103	**1.10**	(c) 0.20		0.003	0.04	**-0.21**
L105	**1.21**		0.27	0.05	0.10	**0.07**
L108	**0.81**		(e) 0.05	0.01	0.10	**-1.10**
L109	**1.40**		(d) (e) 4.63	0.045	0.10	**0.49**
L110	**28.00**	(b) (c) 0.10		(g) 25	(g) 0.1	**9.16**
L111	**1.26**		(d) 22.00	0.0069	0.10	**0.18**

Table 3. z-scores for event 40-3-2 soybean powder level 2 for results reported in m/m %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, z-score calculated on the basis of the robust mean, * no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (e) *U* seems to be underestimated, (f) *U* seems to be overestimated, (g) LOD/LOQ was reported in an inconsistent way, (h) LOD/LOQ seems to be reported in absolute copy numbers, (i) LOD/LOQ seems to be overestimated. Results are as submitted by participants.

Soybean event 40-3-2

Laboratory number	Robust mean = 3.52 m/m %					
	Value	Uncertainty		LOD m/m	LOQ m/m	z-score
		relative	absolute			
L002	**3.05**		(b) 2.28	0.01	0.10	**-0.41**
L005	**3.58**		(a) 1.00	0.027	0.056	**0.05**
L006	**2.85**	(b) (c) (e) 0.05		0.02	0.06	**-0.61**
L007	**3.78**		1.71	0.018	0.02	**0.21**
L009	**4.42**	(b) 30.00		<0.10	<0.10	**0.66**
L011	**4.48**	(a) 8.73		0.05	0.10	**0.70**
L013	**2.61**	(a) (c) 0.54		(g) 0.01	(g) 0.10	**-0.86**
L014	**3.18**		2.01	0.04	0.16	**-0.29**
L015	**4.12**	(a) (c) 2.39		0.10	0.10	**0.46**
L016	**3.46**		(a) 0.71	0.02	0.10	**-0.05**
L017	**3.55**		1.35	0.01	0.03	**0.03**
L019	**3.37**	(a) (c) 1.01		0.04	-	**-0.12**
L020	**4.47**	(a) (c) (e) 0.23		0.045	0.10	**0.70**
L021	**2.86**		0.87	0.045	0.09	**-0.60**
L022	**2.75**		0.51	-	-	**-0.71**
L023	**3.80**	(a) 15.00		-	0.10	**0.23**
L024	**3.36**	(c) 0.35		0.05	0.10	**-0.13**
L027	**3.67**		0.27	0.05	0.10	**0.12**
L030	**3.40**	(c) 1.00		0.001	0.03	**-0.10**
L031	**3.15**		1.09	0.04	0.11	**-0.32**
L032	**4.13**		(a) 1.34	0.50	0.50	**0.47**
L033	**2.90**		0.66	0.01	0.12	**-0.56**
L034	**> 5**	-	-	0.01	0.10	*****
L036	**3.53**		1.16	0.023	-	**0.01**
L037	**3.38**	(b) (c) 0.68		0.05	0.10	**-0.11**
L038	**2.63**	(c) 0.74		0.003	0.04	**-0.84**
L039	**3.07**		(b) 0.37	0.02	0.10	**-0.39**
L041	**3.93**		-	(g) 0.10	(g) 0.10	**0.32**
L042	**4.49**		(e) 0.21	0.05	0.10	**0.71**
L043	**3.24**		0.67	0.01	0.04	**-0.24**
L044	**3.01**	(b) (c) 0.38		-	-	**-0.45**
L045	**3.27**		(a) 0.66	-	0.10	**-0.21**
L046	**3.52**		1.29	0.01	0.10	**0.00**
L047	**4.32**		(a) 0.64	0.04	0.08	**0.60**
L048	**3.78**	(a) 40.00		<0.10	0.10	**0.21**
L050	**3.58**	(c) (e) 0.33		0.10	0.10	**0.05**
L051	**5.02**		(a) 0.05	-	-	**1.03**
L053	**3.12**	(c) 1.50		0.05	0.10	**-0.35**
L054	**3.34**		1.00	0.10	0.10	**-0.15**
L055	**3.50**		1.10	0.04	0.10	**-0.01**
L056	**3.26**	(c) 0.51		(g) -1.00	(g) -1.00	**-0.22**
L059	**2.80**		0.34	0.10	0.40	**-0.66**
L061	**3.66**		1.11	0.01	0.10	**0.12**
L062	**6.20**		(e) 0.28	0.045	0.09	**1.64**
L063	**3.72**		1.00	0.03	0.07	**0.16**
L064	**3.82**		(e) 0.18	0.02	0.06	**0.24**
L065	**4.08**		(d) 13.48	0.045	0.10	**0.43**
L067	**2.78**	-	-	0.045	0.09	**-0.68**
L068	**3.52**		(a) 2.23	0.05	0.05	**0.00**
L072	**3.45**	(a) 32.98		0.10	0.10	**-0.05**
L074	**3.81**		(a) 0.43	0.10	0.29	**0.23**
L076	**3.00**	-	-	0.10	-	**-0.46**
L078	**3.07**		2.22	0.002	0.018	**-0.39**
L079	**4.91**	(a) (c) (e) 0.27		0.04	0.13	**0.96**
L080	**3.46**	(c) 2.20		0.01	0.06	**-0.05**
L082	**1.00**		(a) (e) 0.06	0.10	0.10	**-3.64**
L084	**4.25**		(a) 0.98	0.03	0.10	**0.55**
L086	**4.60**		(a) 0.90	0.05	0.10	**0.78**
L089	**3.45**		0.71	0.02	0.10	**-0.05**
L092	**3.33**	-	-	0.007	0.013	**-0.16**
L093	**3.70**	(b) (c) 0.46		0.01	0.10	**0.15**
L095	**4.14**	-	-	0.01	0.10	**0.47**
L097	**3.24**		1.16	(i) 0.51	(i) 1.15	**-0.24**
L098	**6.04**	(c) (e) 0.09		(h) 45	(h) 1	**1.57**
L101	**3.68**		(e) 0.17	0.03	0.10	**0.13**
L103	**2.90**	(c) 0.80		0.003	0.04	**-0.56**
L105	**3.76**		0.48	0.05	0.10	**0.19**
L108	**2.70**		(e) 0.10	0.01	0.10	**-0.76**
L109	**3.96**		(d) 13.08	0.045	0.10	**0.34**
L111	**3.58**		(d) 22.00	0.0069	0.10	**0.05**

Table 4. z-scores for event 40-3-2 soybean powder level 1 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, z-score calculated on the basis of the robust mean, * no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (e) *U* seems to be underestimated, (f) *U* seems to be overestimated, (g) LOD/LOQ was reported in an inconsistent way, (h) LOD/LOQ seems to be reported in absolute copy numbers, (i) LOD/LOQ seems to be overestimated. Results are as submitted by participants.

	Soybean event 40-3-2					
Laboratory number	**Robust mean = 1.05 cp/cp %**					
	Value	**Uncertainty**		**LOD cp/cp**	**LOQ cp/cp**	**z-score**
		relative	**absolute**			
L001	**1.10**	9.43		0.009	0.018	**0.13**
L003	**1.18**		0.39	0.03	0.07	**0.34**
L004	**0.87**		0.23	0.14	0.58	**-0.54**
L008	**1.15**		(b) 0.19	0.045	0.09	**0.26**
L009	**1.31**	30.00		0.02	0.07	**0.64**
L010	**1.08**		(e) 0.08	0.02	0.05	**0.08**
L012	**1.00**	28.00		-	-	**-0.14**
L018	**1.02**	(c) 0.13		0.01	0.045	**-0.08**
L020	**0.95**	(a) (c) 0.22		0.045	0.10	**-0.29**
L025	**1.59**		(a) 0.65	-	-	**1.20**
L026	**1.30**	(c) 0.90		0.08	0.31	**0.62**
L028	**1.55**	(f) 100.00		(g) 0.10	(g) 0.10	**1.13**
L029	**1.80**		(a) 0.30	0.03	0.05	**1.56**
L035	**0.90**		0.30	0.01	0.10	**-0.45**
L040	**0.58**	(a) (c) 0.34		0.01	0.05	**-1.72**
L049	**1.03**		(b) 0.32	0.05	0.10	**-0.05**
L052	**0.98**		0.12	-	0.01	**-0.20**
L056	**0.71**	(c) 0.23		(g) -1.00	(g) -1.00	**-1.13**
L057	**0.86**		0.65	0.10	0.30	**-0.58**
L058	**0.96**		0.15	0.10	0.40	**-0.26**
L066	**0.85**	(a) (c) (e) 0.09		0.04	0.08	**-0.61**
L069	**1.11**	27.54		0.10	0.10	**0.16**
L070	**0.83**		(a) 0.21	0.01	0.05	**-0.68**
L071	**0.51**		0.28	-	-	**-2.09**
L081	**1.92**	-	-	0.01	0.10	**1.75**
L083	**1.05**		0.26	0.09	0.017	**0.00**
L084	**1.60**		(a) 0.66	0.02	0.10	**1.22**
L085	**1.86**	(c) 0.95		(g) 0.0022	(g) 0.0175	**1.66**
L087	**0.96**	(a) 20.00		(g) 0.05	(g) 0.10	**-0.26**
L088	**1.08**		(d) 26.54	-	-	**0.07**
L090	**0.70**		0.40	0.01	0.10	**-1.17**
L091	**1.25**	(a) 12.60		0.05	0.10	**0.51**
L094	**1.17**	(b) (c) 0.18		0.005	0.05	**0.31**
L099	**0.31**		0.10	0.10	0.10	**-3.53**
L102	**0.03**	(a) (c) (e) 0.02		0.02	0.10	**-10.29**
L104	**1.65**	-	-	-	-	**1.31**

Table 5. z-scores for event 40-3-2 soybean powder level 2 for results reported in cp/cp %. LOD = Limit of Detection, LOQ = Limit of Quantification, - = not reported, z-score calculated on the basis of the robust mean, * no z-score attributed, (a) Uncertainty (*U*) was reported in an inconsistent manner, (b) *U* was reported in an incomplete manner, (c) *U* seems to be an absolute value, (d) *U* seems to be a relative value, (e) *U* seems to be underestimated, (f) *U* seems to be overestimated, (g) LOD/LOQ was reported in an inconsistent way, (h) LOD/LOQ seems to be reported in absolute copy numbers, (i) LOD/LOQ seems to be overestimated. Results are as submitted by participants.

Soybean event 40-3-2						
Laboratory number	**Robust mean = 3.07 cp/cp %**					
	Value	**Uncertainty**		**LOD cp/cp**	**LOQ cp/cp**	**z-score**
		relative	**absolute**			
L001	**3.20**	9.86		0.01	0.019	**0.12**
L003	**3.08**		1.02	0.03	0.07	**0.01**
L004	**2.40**		0.40	0.15	0.61	**-0.71**
L008	**3.52**		(b) 0.58	0.045	0.09	**0.40**
L009	**4.03**	30.00		0.02	0.07	**0.79**
L010	**3.47**		(e) 0.13	0.02	0.05	**0.36**
L012	**2.80**	26.00		-	-	**-0.27**
L018	**3.61**	(c) (e) 0.15		0.01	0.045	**0.47**
L020	**2.72**	(a) (c) 0.59		0.045	0.10	**-0.35**
L025	**3.52**		(a) 1.44	0.10	0.10	**0.40**
L026	**2.80**	(c) or (e) 2.20		0.03	0.13	**-0.27**
L028	**2.54**	(f) 100.00		(g) 0.10	(g) 0.10	**-0.55**
L029	**4.33**		(a) 0.70	0.03	0.04	**1.00**
L035	**3.20**		0.90	0.01	0.10	**0.12**
L040	**2.83**	(a) (c) 0.34		0.01	0.05	**-0.23**
L049	**3.38**		(b) 0.73	0.05	0.10	**0.28**
L052	**3.16**		(e) 0.16	-	0.02	**0.08**
L056	**2.95**	(c) (e) 0.16		(g) -1.00	(g) -1.00	**-0.11**
L057	**2.89**		1.74	0.10	0.30	**-0.17**
L058	**2.83**		(e) 0.07	0.10	0.40	**-0.23**
L066	**2.92**	(a) (c) 0.30		0.04	0.08	**-0.14**
L069	**3.49**	27.54		0.10	0.10	**0.37**
L070	**2.22**		(a) 0.49	0.01	0.05	**-0.94**
L071	**1.24**		0.37	-	-	**-2.62**
L081	**6.97**	-	-	0.01	0.10	**2.37**
L083	**2.31**		0.85	0.09	0.017	**-0.82**
L084	**4.25**		(a) 0.98	0.03	0.10	**0.94**
L085	**7.69**	(c) or (e) 2.59		(g) 0.0011	(g) 0.0085	**2.66**
L087	**2.90**	(a) 20.00		(g) 0.05	(g) 0.10	**-0.16**
L088	**2.83**		(d) 9.98	-	-	**-0.23**
L090	**2.00**		0.60	0.01	0.10	**-1.24**
L091	**3.84**	(a) 22.60		0.05	0.10	**0.65**
L094	**4.02**	(b) (c) 0.71		0.005	0.05	**0.78**
L099	**0.43**		(e) 0.1	0.10	0.10	**-5.69**
L102	**0.24**	(a) (c) (e) 0.10		0.02	0.10	**-7.38**
L104	**3.94**	-	-	-	-	**0.72**

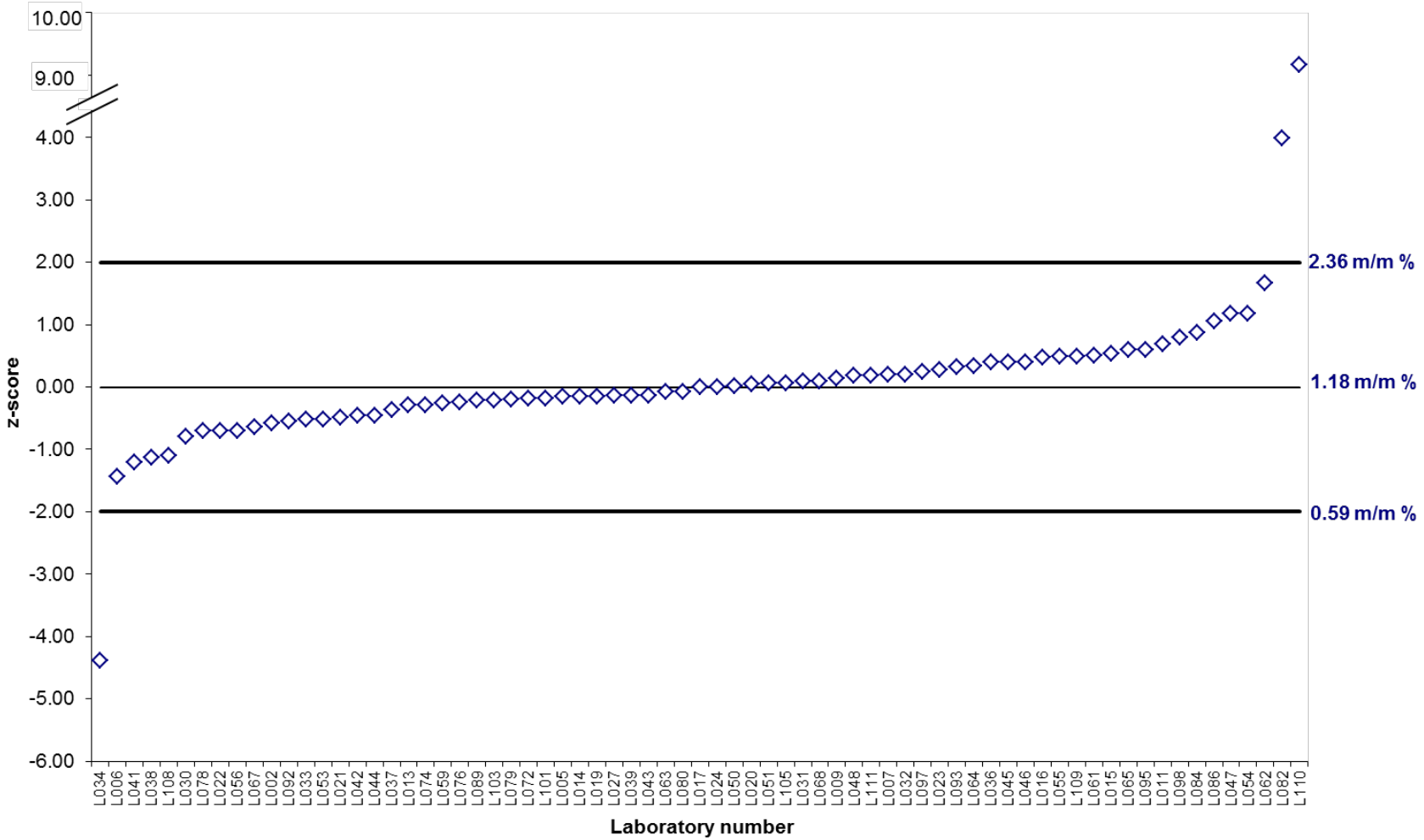

Figure 5. z-scores for soybean event 40-3-2 powder level 1 on the basis of a robust mean of 1.18 m/m % (◊)

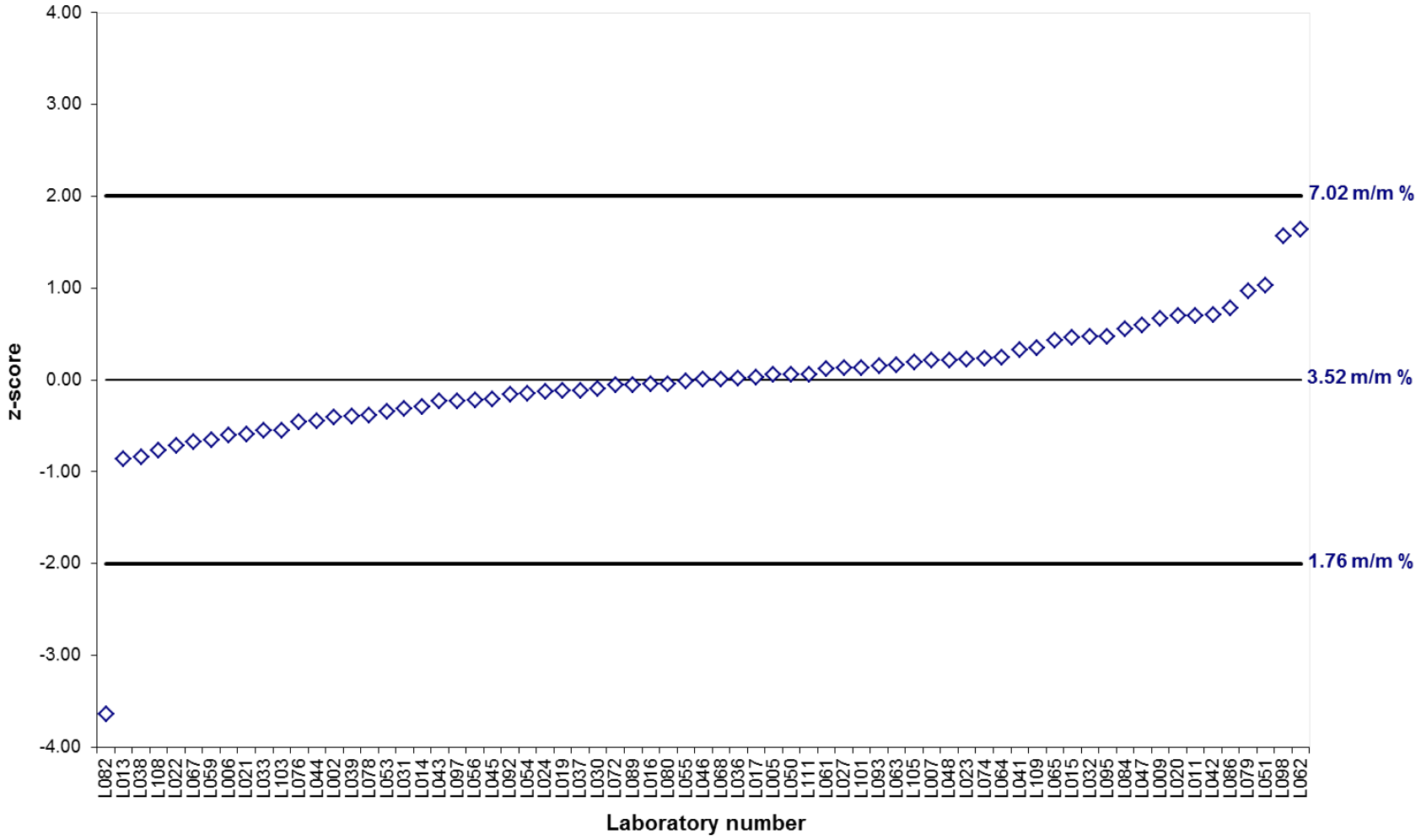

Figure 6. z-scores for soybean event 40-3-2 powder level 2 on the basis of a robust mean of 3.52 m/m % (◊)

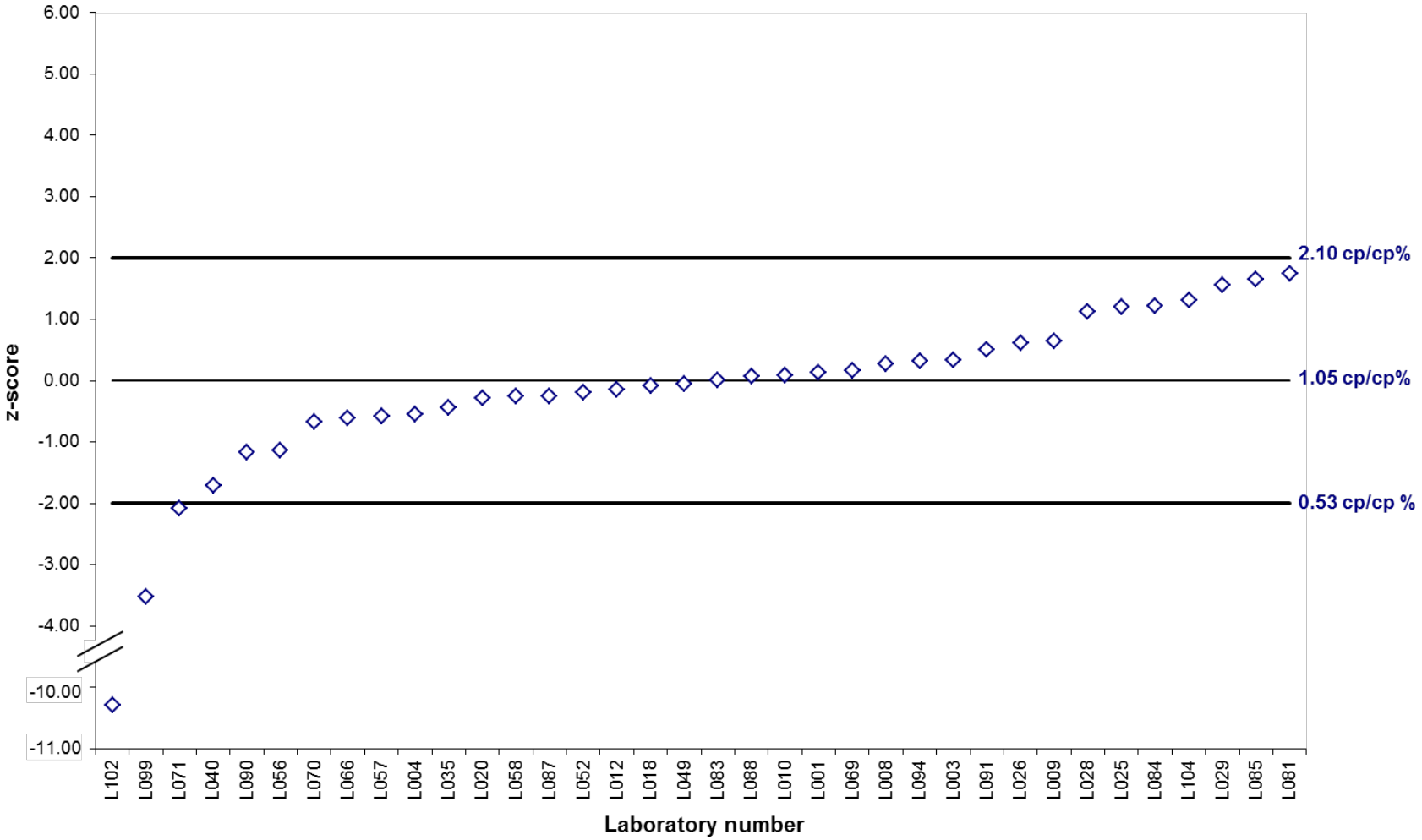

Figure 7. z-scores for soybean event 40-3-2 soybean powder level 1 on the basis of a robust mean of 1.05 cp/cp % (◊)

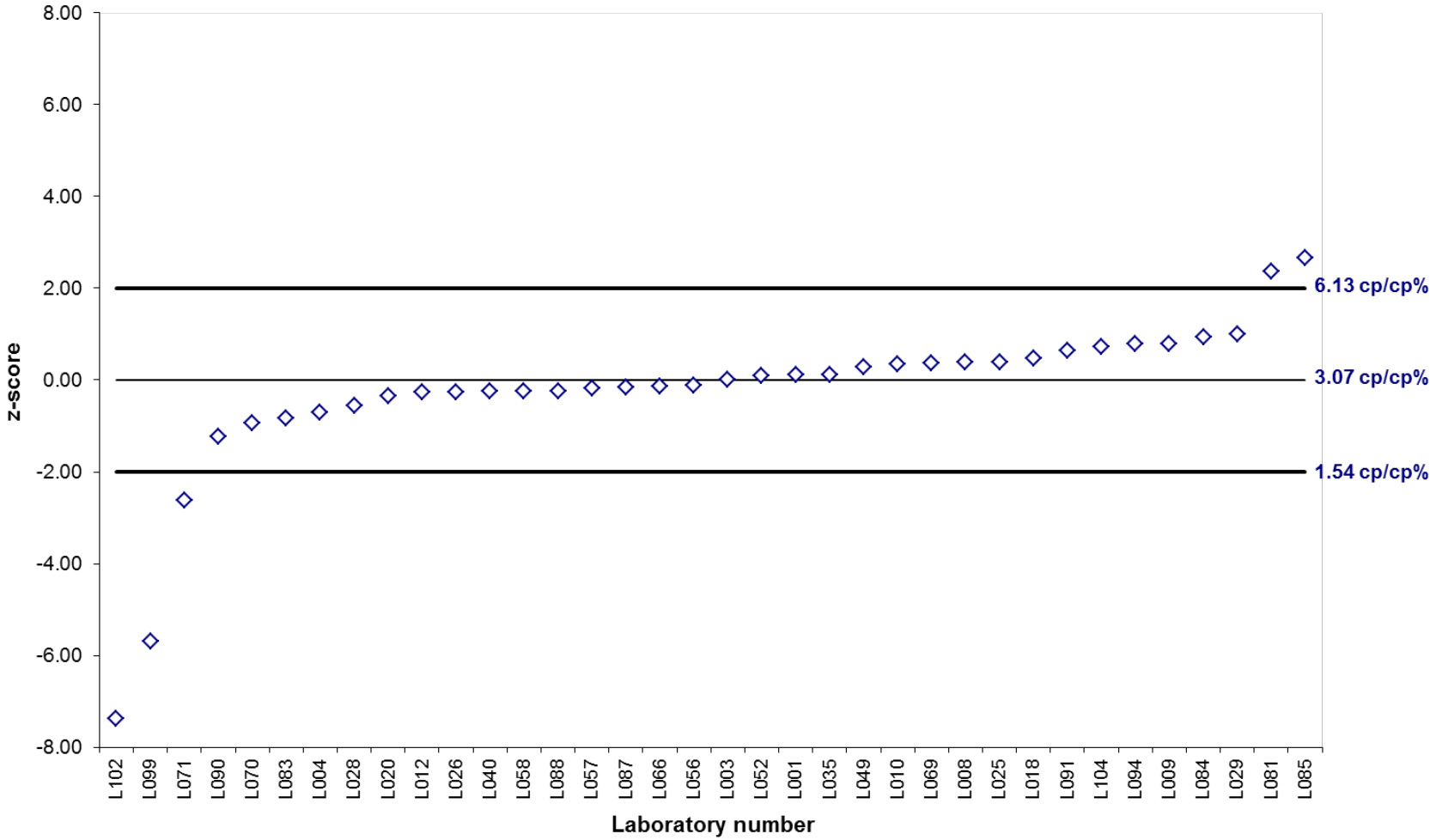

Figure 8. z-scores for soybean event 40-3-2 soybean powder level 2 on the basis of a robust mean of 3.07 cp/cp % (◊)

6. Interpretation of z-scores

In general one assumes a normal distribution when calculating z-scores. In which case there is a 5 % probability that some z-scores will fall outside the working range of -2 to +2 and a 0.3 % probability that some z-scores will fall outside the working range of -3 to +3. A z-score outside the working range of -2 to +2 indicates that a participant is probably not performing according to specifications although this cannot be stated with 100 % certainty. The higher the value of the standard deviation for comparative testing $\hat{\sigma}$, the more likely participants with a z-score outside the working range of -2 to +2 are underperforming. However, a greater $\hat{\sigma}$ will also increase the probability of accepting unsatisfactory measurement results. Hence, a compromise should be made between the choice of the value of $\hat{\sigma}$ and the attempt to assess the participants' performance. In any case a z-score outside the working range of -3 to +3 will quite clearly identify an underperforming participant and will require follow-up. It should be taken into consideration that a laboratory performing well has a 5 % probability of obtaining a z-score outside the working range of -2 to +2 by mere chance.

7. Evaluation of results

In this third comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for both soybean powder levels 1 and 2 regardless of the calibration method and the measurement unit.

An overview of the laboratories having obtained outlying z-scores is provided in Table 6.

Table 6: Overview of laboratories with outlying z-scores for the soybean powder levels 1 and 2 test items in m/m % and in cp/cp %. * no z-score was attributed because the laboratory reported the GM content as > value *x*; - = no results reported

	Outlying z-scores			
Laboratory	**[m/m %]**		**[cp/cp %]**	
number	**Level 1**	**Level 2**	**Level 1**	**Level 2**
L034	x	*		
L071			x	x
L081				x
L082	x	x		
L085				x
L099			x	x
L102			x	x
L110	x	-		

A higher proportion of laboratories obtained a z-score outside the range of -2 to +2 for the results expressed in cp/cp %. The causes for the outlying z-scores were investigated on the basis of raw data provided by the laboratories and are summarised in Table 7. Due to

technical problems with the real-time PCR equipment L102 and L110 could not submit their raw data.

Table 7: Overview of the possible reasons for outlying z-scores. Ct value = cycle threshold value, NTC = no template control, R^2 = coefficient of determination.

Laboratory number	Positive NTC	Problems with the calibration curve	Slope outside range	R^2 outside range	Ct values outside the working range	Low passive reference ROX signal	Swapped results
L034	x	x	x				
L071		x		x	x		
L081	x					x	
L082							x
L085		x			x		
L099		x		x	x		

In this section the terms used in Table 7 are further explained.

- 'Positive NTC' (i.e. no template control) means that amplification was noted for the negative control.
- 'Problem with calibration curve' refers to the standards of the dilution series, in that the measured Ct diverged from the extrapolated Ct value[17]
- 'Slope outside range' indicates that the slope of the calibration curve was poor compared to the acceptable values ($-3.6 \leq$ slope ≤ -3.1) outlined in the ENGL guidance[17].
- 'R^2 outside range' implies that the coefficient of determination (R^2) was poor compared to the acceptable value ($R^2 \geq 0.98$) outlined in the ENGL guidance document[17].
- 'Ct values outside working range' means that the Ct values of the unknown samples fell beyond the linear working range of the calibration curve. Since it is not known if the calibration curve shows a linear pattern beyond its working range, it is unacceptable to extrapolate the quantification of unknown samples beyond the working range of the calibration curve.
- 'Low passive reference ROX signal' refers to the very low signal of the passive reference ROX around the edges of the real-time PCR plate which could indicate an evaporation problem.

- 'Swapped results' means that the participant has swapped the results reported for the soybean powder levels 1 and 2 test items.

8. Performance of laboratories

Given the legal mandate of the EU-RL GMFF to organise comparative testing for NRLs and ensure an appropriate follow-up of their performance, section 8.1 focuses on the performance of NRLs. However, the performance of other participants is also monitored and they also receive suggestions to improve their performance when needed (section 8.2).

8.1 NRLs

The third comparative testing round showed an overall positive performance of the participating NRLs.

Seventy-one NRLs were invited to participate in this comparative testing round. Eight NRLs declined participation of which one declared no longer to be nominated as a NRL. One (L077) out of 62 NRLs that registered for the third comparative testing round did not report any results. The NRL gave no reason for not reporting results.

Two (L071 and L081) out of 61 NRLs, obtained z-scores outside the working range of -2 to +2. Both laboratories expressed the results in cp/cp %. Analysing the raw data of those participants allowed identifying possible causes for these results.

A number of observations were made regarding the GM system of L071. The standard curve was only composed of three points. The coefficient of determination ($R^2 = 0.93$) of the calibration curve for the GM target was poor compared to the value ($R^2 \geq 0.98$) outlined in the ENGL guidance[17] document. Several Ct values of the unknown samples and quality control materials fell outside the linear working range of the calibration curve. Since it is not known if the calibration curve shows a linear pattern beyond its working range, it can never be accepted to extrapolate the quantification of unknown samples beyond the working range of the standard curve. For the reference system the measured Ct diverged from the extrapolated Ct value. A slight contamination was noted for the extraction control of the reference system of L081 (Ct = 43.56). In addition, the signal of the passive reference ROX was very low around the edges of the real-time PCR plate which could indicate an evaporation problem.

Both NRLs were asked to repeat the experimental work related to this third comparative testing round. Before the shipment of a new set of test items advice was provided regarding the approach to be followed for the experimental analyses. The advice was in line with the observations noted in Table 7 for each participant.

In addition, some observations were made regarding the reporting of the Limit of Detection (LOD) and Limit of Quantification (LOQ). L013 reported results in m/m % whereas the LOD and LOQ were reported in cp/cp % (Tables 2 and 3). L028 reported results in cp/cp %

whereas the LOD and LOQ were reported in m/m % (Tables 4 and 5). Most likely it concerns a reporting mistake but the LOD and LOQ values have been denoted as 'inconsistent reporting'.

The NRL that was visited by two staff members of the EU-RL GMFF in April 2011 as a result of the underperformance in the first two comparative testing rounds, has drastically improved its performance in the current comparative testing round. This NRL (L111) obtained z-scores of 0.18 and 0.05 for soybean powder levels 1 and 2 respectively, in this comparative testing round.

8.2 Non-NRLs

Six (L034, L082, L085, L099, L102 and L110) out of 40 non-NRLs, obtained z-scores outside the working range of -2 to +2. Three (L085, L099 and L102) of those laboratories had expressed the results in cp/cp %. Three laboratories (L034, L082 and L110) had expressed the results in m/m %. Analysing the raw data of those participants allowed identifying possible causes for these results. Due to technical problems with the real-time PCR equipment L102 and L110 could not submit their raw data.

The reference system of L034 showed a contamination of the extraction blank and the blank of the real-time PCR master mix. Although L034 used a delta Ct method, the EU-RL GMFF used the raw data to calculate the slope of the GM system. The slope (-2.69) was poor compared to the values ($-3.6 \leq$ slope ≤ -3.1) outlined in the ENGL guidance[17] document. In addition, there was a problem with the standards of the dilution series, in that the measured Ct diverged from the extrapolated Ct value[17]. In the case of L082 it was suspected that the laboratory had swapped the values reported for soybean powder levels 1 and 2. L085 seemed to have a problem with the dilution series of the standard curve corresponding to the GM system. The measured Ct values of the unknown samples diverged from the theoretical Ct values[17]. In addition, several Ct values of the unknown samples were outside the linear working range of the standard curve. In the case of L099 several problems were noted. The calibration curve was composed of four points and only one PCR replicate per concentration. The coefficient of determination ($R^2 = 0.96$) of the calibration curve for the GM target was poor compared to the value ($R^2 \geq 0.98$) outlined in the ENGL guidance[17] document. In addition, the measured Ct diverged from the extrapolated Ct value[17]. For the reference system the Ct values of the unknown samples fell outside the linear working range of the calibration curve.

Those six laboratories were asked to repeat the experimental work related to this third comparative testing round. Before the shipment of a new set of test items advice was provided regarding the approach to be followed for the experimental analyses. The advice was in line with the observations noted in Table 7 for each participant.

Some observations were made regarding the reporting of the LOD and LOQ. L041 reported results in m/m % whereas the LOD and LOQ were reported in cp/cp % (Tables 2 and 3). L085 and L087 reported results in cp/cp % whereas the LOD and LOQ were reported in m/m % (Tables 4 and 5). Most likely it concerns a reporting mistake but the LOD and LOQ

values have been denoted as 'inconsistent reporting'. L097 seems to have overestimated the LOD and LOQ (Tables 2 and 3). L110 seems to have reported the LOD in absolute copy numbers and the LOQ in m/m % (Table 2).

8.3 Results repetition experimental work

The results of the repetition of the experimental work are depicted in Table 8. Participants with outlying z-scores were asked to repeat the experimental work. All laboratories except L085 and L102 repeated the experimental work and submitted results within the deadline (Table 8). L102 could not repeat the experimental work due to technical problems with the real-time PCR instrument. L085 did not give a reason for not repeating the experimental work.

Table 8. Repetition of experimental work: reported results in m/m % (a) and in cp/cp % (b) and z-scores for event 40-3-2 soybean powder levels 1 and 2. z-scores were calculated on the basis of the robust mean. Results are as submitted by participants.

a) Soybean event 40-3-2

Laboratory number	Robust mean = 1.17 m/m %		
	Value	**Uncertainty**	**z-score**
L034	**0.86**	0.25	**-0.90**
L081	**1.47**	-	**0.65**
L082	**1.00**	0.30	**-0.46**
	Robust mean = 3.53 m/m %		
L034	**2.96**	0.88	**-0.51**
L081	**4.24**	-	**0.53**
L082	**3.70**	0.06	**0.13**

b) Soybean event 40-3-2

Laboratory number	Robust mean = 1.06 cp/cp %		
	Value	**Uncertainty**	**z-score**
L071	**1.38**	26.4	**0.76**
L099	**0.67**	0.10	**-1.33**
	Robust mean = 3.07 cp/cp %		
L071	**2.90**	28.87	**-0.17**
L099	**0.62**	0.10	**-4.64**

8.3.1 NRLs

Both NRLs (L071 and L081) that repeated the experimental work obtained satisfactory z-scores upon repetition of the experimental work (Table 8). L071 expressed the repeated results again in cp/cp % whereas L081 expressed the results of the repeated analyses in m/m %.

8.3.2 Non-NRLs

With the exception of L099 and L110, the non-NRLs (L034 and L082) that repeated the experimental work obtained satisfactory z-scores upon repetition of the experimental work (Table 8). L099 reported almost identical values for soybean powder GM levels 1 and 2. The laboratory was again asked to submit its raw data. L110 did not report any values for the repetition of the experimental work but provided the raw data. Several problems were encountered such as calibration curves composed of three points, single PCR replicates for standards and unknowns, and a R^2 coefficient of 0.85. It is quite clear that this laboratory would benefit from a training course on GMO analysis organised by the EU-RL GMFF.

9. Conclusions

In this third comparative testing round participants were asked to determine the GM content in two test items containing different GM percentages of soybean event 40-3-2. Both test items were produced by the EU-RL GMFF.

Results could be reported in either m/m % or cp/cp %. The majority of participants submitted the results in m/m %. A few participants submitted the results in cp/cp % using a plasmid DNA calibrant (N = 6). Since it is not good practice to calculate the robust mean on a limited number of data, all results expressed in cp/cp % were pooled irrespective of the DNA calibrant used. However, the EU-RL GMFF is aware that differences due to the nature of the calibrant used can be observed[18]. Two laboratories (L071 and L081) using a plasmid DNA calibrant obtained a z-score outside the working range of -2 to +2. In the case of L071 the standard curve was only composed of three points whereas L081 experienced problems with the passive reference ROX.

There was a disparity observed between the measured GM content and the GM levels prepared through weighing. The reasons for this are unclear. However, during the preparation of test items a difference in mass per seed was observed between the GM and conventional seeds that originated from different lines. In the literature it has been shown that the DNA density of any specific cultivar, whether or not transgenic, cannot be automatically assumed to be proportional to the mass ratio of that cultivar in the soybean mixture[19]. Therefore, this may have led to a higher GM content in the test items than expected.

Although soybean event 40-3-2 is homozygous for the GM target, a difference was noted between results expressed in mass/mass % and in cp/cp % (1.18 m/m % versus 1.05 cp/cp % and 3.52 m/m % versus 3.07 cp/cp % for soybean powder levels 1 and 2). One can only speculate about the reason for this difference. When using a dual target plasmid calibrant for calibration one can be quite sure that the ratio of the transgenic target to the endogenous target is equal to one. When using genomic DNA for calibration the endogenous target is supposed to be a single copy gene. The reality however shows a 12 % variation of the nuclear DNA content in soybean plants[20]. The presence of the endogenous gene in a low copy number instead of a single copy number will undoubtedly have an impact on the GM % obtained.

In this third comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for both soybean powder levels 1 and 2 regardless of the calibration method and the measurement unit. Eight laboratories obtained a z-score outside the working range of -2 to +2. The performance of these laboratories will be monitored in future comparative testing rounds. If necessary, on-site visits to those participants could be foreseen to provide assistance.

Since only about 56 % of participants provided information on measurement uncertainty (MU) in a complete and consistent manner, there is a need to provide laboratories with guidance and training to harmonise the MU reported in the field of GMO detection. For the comparative testing round ILC-EURL-GMFF-CT-02/11 participants have been provided with a guidance document for the estimation of the measurement uncertainty.

Participants' assessment of results in relation to MU needs to be improved. This will have an impact on the enforcement of the 0.9 % threshold. Regulation (EU) No 619/2011 [21] lays down rules for reporting the outcome of the analysis as $x \pm U$ whereby x is the analytical result measured for one GM event and U is the appropriate expanded measurement uncertainty. Regulation (EC) No 1829/2003[1] establishes a threshold for labelling of food and feed products consisting of or containing more than 0.9 % GMOs. Labelling is necessary in case the reported value x minus the expanded uncertainty U is equal to or above 0.9 % GM. This approach was followed to establish the percentage of participants in this comparative testing round whose decision would be to label the soybean powder level 1 test item as containing GM. For 52 % of participants who reported the results in m/m % and 31 % of participants who reported the results in cp/cp % the decision would be to label the soybean powder level 1 test item. The robust means and expanded uncertainties of the soybean powder level 1 test item calculated on the basis of the data of all participants are 1.18 ± 0.09 m/m % and 1.05 ± 0.14 cp/cp % (Table 1). For both measurement units the subtraction of the expanded uncertainty from the robust means would give rise to a value above 0.9 % GM and thus the decision would be to label the soybean powder level 1 test item.

The EU-RL GMFF is aware of the fact that the laboratories performing the analyses in most cases do not decide on the labelling of the product. It is the responsibility of the laboratories involved in official control to issue a test report including a statement regarding the expanded measurement uncertainty.

The observation that the implementation of the labelling rules described in Regulation (EU) No 619/2011 would have resulted in the labelling of 52 % of all results expressed in m/m % and 31 % of the results expressed in cp/cp % highlights the importance of the correct estimation of the expanded measurement uncertainty. Indeed the ISO 17025[22] Standard states that test reports 'shall where applicable include a statement on the estimated uncertainty of measurement when it is relevant to the validity or application of the test results or when the uncertainty affects compliance to a specification limit'.

The European legislation requires all NRLs appointed under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006 to be accredited under ISO 17025. The observations regarding the reporting of the measurement uncertainty and the decision regarding the labelling of the soybean powder level 1 test item demonstrate the need for guidance in order to obtain a correct implementation of the labelling threshold of 0.9 %.

10. References

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22. ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories

11. Questionnaire data

The total number of answers in the questionnaire to each question does not always correspond to the total number of reported results. This is due to the fact that some questions were not answered by the participants.

1. DNA extraction method?	**No. of laboratories**
a) ISO validated	35
b) EU-RL validated	9
c) National reference method	2
d) International literature	6
e) In-house developed and optimised	17
f) **Other of which**	35
Commercial kit	2
Congen SureFood Prep Plant X-Kit	1
CTAB + Wizard	1
EU-RL validated CTAB method with minor modifications	1
Fast ID Genomic DNA Extraction Kit Instruction Manual	1

Gene elute plant - Sigma	1
GeneScan GENESpin	4
JRC course The analysis of Food Samples for the Presence of Genetically Modified Organisms, Session 4, Extraction and Purification of DNA	1
Macherey Nagel Nucleospin	7
Modified version of Promega developed extraction method	1
Promega Wizard	1
Qiagen DNeasy plant mini kit	2

1.3. Was the DNA extraction method used within the scope of your ISO/IEC 17025 accreditation?	**No. of laboratories**
a) Yes	85
b) No	19

2. Number of replicate DNA extractions from test material?	**No. of laboratories**
a) 2	77
b) 3	15
c) 4	7
d) **Other of which**	5
6	2
7	1
10	2

3. Sample intake (in g) for the DNA extraction?	**No. of laboratories**
a) < 0.1	5
b) 0.1-0.2	66
c) > 0.2	19
d) **Other of which**	14
0.02	1
0.18	1
0.50	1
0.70	1
1	7
2	2
3	1

4. DNA extraction method/kit used?	**No. of laboratories**
a) CTAB	35
b) CTAB-derived	15
c) Biotecon	2
d) GeneScan GENESpin	8

e) Guanidine HCl with proteinase K	5
f) Macherey Nagel Nucleospin	14
g) Promega Wizard	6
h) Qiagen DNeasy plant mini kit	10
i) TEPNEL kit	1
j) Proprietary method	1
k) **Other of which**	8
Congen SureFood Prep Kit	2
Dellaporta derived Method	1
E.Z.N.A. Plant DNA kit Omega	1
Fast ID Genomic DNA Extraction Kit	1
Gene elute plant - Sigma	1
Genescan DNAExtractor (Clean)	1
Modified DNeasy Blood and Tissue Kit	1

5. How was the clean-up of the DNA performed?	**No. of laboratories**
a) No DNA clean-up	53
b) Ethanol precipitation	15
d) Promega Wizard DNA clean-up resin	12
e) Qiagen QIAQuick	6
f) Qiagen Genomic-Tip 20/G	1
g) Silica	7
h) Proprietary method	1
i) **Other of which**	9
Eurofins GeneScan Cleaning Columns	1
Genespin	1
Promega Wizard SV Genomic DNA purification system (in-house modified)	1
Promega Wizard resin + Qiagen QIAQuick	1
Qiagen DNeasy Plant Mini Kit	1
Qiagen QIAmp DNA minikit	1
Sigma GenElute Clean up Kit	2
Zymo Research DNA Clean & Concentrator	1

6. How have you quantified the DNA?	**No. of laboratories**
a) Gel	4
b) UV spectrophotometer	49
c) Nanodrop	29
d) Fluorometer	14
e) Not applicable (i.e. DNA was not quantified)	2
f) **Other of which**	6
Biophotometer Plus (Eppendorf)	2
Estimation was made using Real-time PCR	2
Nanovue GE	2

7. What was the DNA concentration (in ng/µL) of the undiluted extracted sample?	**No. of laboratories**
a) 0-50	17
a) 0-50, b) 50-100	1
a) 0-50, b) 50-100, c) 100-150	3
b) 50-100	20
b) 50-100, c) 100-150	2
b) 50-100, c) 100-150, d) 150-200	1
c) 100-150	9
c) 100-150, d) 150-200	4
d) 150-200	3
d) 150-200, e) 200-250	5
e) 200-250	5
f) 250-300	2
g) 300-350	3
g) 300-350, h) 350-400	4
h) 350-400	1
h) 350-400, i) 400-450	1
h) 350-400, j) 450-500	1
i) 400-450	3
j) 450-500	3
k) 500-550, t) 950-1000	1
n) 650-700	1
r) 850-900	2
t) 950-1000	3
u) **Other of which**	6
1200	1
1400	2
1500	1
1520	1
2000	1

7.1. Dilution factor?	**No. of laboratories**
a) 0.2	1
b) 5	1
c) 8	1
d) 10	1

8. Dilution buffer?	**No. of laboratories**
a) TE (10 mM Tris-HCl, 1 mM EDTA)	18
b) TE 0.1X (10 mM Tris-HCl, 0.1 mM EDTA)	12
c) TE low (1 mM Tris, 0.01 mM EDTA)	1
d) Water	64
e) **Other of which**	9
5 mM Tris-HCl, pH 8.5	1
5 mM Tris-HCL, pH 8.8	1

AE buffer (Qiagen)	2
No dilution applied	1
TE (10 mM TrisHCl, 0,2 mM EDTA)	1
TE 0.2X (2 mM Tris-HCl, 0.2 mM EDTA)	2
TE 0.5X	1

9. Validation status of the PCR analytical method?	**No. of laboratories**
a) ISO/CEN published method	32
b) EU-RL validated method for RoundUp Ready soybean	36
c) National reference method	4
d) International literature	9
e) In-house developed and optimised	10
f) **Other of which**	6
Congen SureFood GMO Roundup Ready Soya Kit	1
Eurofins GMO Quant Roundup Ready Soy kit	3
Real-time PCR	1
TaQMan Genetically Modified Organism (GMO) Detection Kits, User Guide, Applied Biosystems, 2001	1

9.3. Was the PCR analytical method used within the scope of your ISO/IEC 17025 accreditation?	**No. of laboratories**
Yes	84
No	20

10. Real-time PCR analytical method	**No. of laboratories**
Multiplex PCR	5
Singleplex PCR	99

11. Real-time PCR instrument?	**No. of laboratories**
a) ABI 7000	3
b) ABI 7300	11
c) ABI 7500	27
d) ABI 7700	4
e) ABI 7900HT	24
f) ABI StepOne & StepOnePlus real-time PCR system	2
g) BioRad icycler	5
h) Corbett Rotor-Gene 6000	2
i) Roche LightCycler 2.0	5
j) Roche LightCycler 480	5
k) Stratagene Mx3000/Mx3005	7

m) **Other of which**	8
ABI 2720	1
ABI 7500 Fast System	2
BioRad CFX96	1
BioRad IQ5	1
Qiagen Rotor-Gene Q (5-plex, HRM)	1
Roche Light cycler 1.0	1
Roche LightCycler (from 1999)	1

11.2. In case of ABI 7900HT 9600 emulation mode ?	**No. of laboratories**
a) Yes	10
b) No	15

11.3. Cycling parameters ?	**No. of laboratories**
10 min 40°C, 10 min 95°C, 50x (5 s 95°C; 15 s 60°C; 12 s 72°C), 60 s 40°C	1
10 min 95°C, 40x (15 s 95ºC; 60 s 60ºC; 31 s 72ºC)	1
10 min 95°C, 45x (10 s 95 ºC; 30 s 60 ºC)	1
10 min 95°C, 45x (15 s 95°C; 60 s 60°C)	8
10 min 95°C, 45x (20 s 95°C; 60 s 60°C)	2
120 s 50 °C, 10 min 95°C, 45x (15 s 95°C; 60 s 60°C)	6
120 s 50 °C, 10 min 95°C, 45x (30 s 95°C; 60 s 60°C)	1
120 s 50°C , 10 min 95°C, 15 s 95°C, 60 s 60°C	1
120 s 50°C , 10 min 95°C, 40x (15 s 95°C; 60 s 60°C)	2
120 s 50°C , 10 min 95°C, 50x (15 s 95°C; 60 s 60°C)	2
120 s 50°C , 10 min 96°C, 45x (20 s 96°C; 60 s 60°C)	1
120 s 50°C, 10 min 95°C, 15 s 95°C; 60 s 55°C	1
120 s 50°C, 10 min 95°C, 15 s 95°C; 60 s 60°C	1
120 s 50°C, 10 min 95°C, 45x (15 s 95°C; 60 s 60°C)	5
120 s 50°C, 10 min 95°C, 45x (30 s 95°C; 60 s 55°C)	1
120 s 50ºC, 10 min 98ºC, 50x (15 s 95ºC; 60 s 60ºC)	1
120 s 94°C, 45x (15 s 94°C;60 s 60°C)	1
15 min 95°C, 20°C/s; 10 s 95°C, 20°C/s; 30 s 60°C, 20°C/s; 30 s 72°C, 2°C/s; 10 s 40°C, 20°C/s	1
15 s 95°C, 60 s 55°C	1
20 s 95°C, 45x (3 s 95°C; 30 s 60°C)	1
3 min 95°C, 80 s 95°C, 80 s 68°C, 20 s 72°C, 3 min 4°C	1
3 min 95°C, 45x (15 s 95°C; 1 min 60°C)	1
45 cycles	1
45x (5 s 95°C; 25 s 60°C)	1
5 min 95°C, 45x (15 s 95°C; 30 s 60°C)	1
5 min 95°C, 10 s 95°C, 15 s 62°C, 30 s 65°C	1
50 cycles	1
50 x 15 min 95°C; 60 s 60°C	1

As per EU-RL protocol	1
Basic Fast mode on ABI 7500 Fast system	2
Different conditions for GMO-specific gene and for the reference gene	1
For 40-3-2 system: 10 min 95ºC; 40 x (15 s 95ºC; 60 s 55ºC); For soybean Lec system: 600 s 95ºC; 45 x (15 s 95ºC; 60 s 60ºC)	1
For 40-3-2 system: 120 s 50ºC; 10 min 95ºC; 45 x (15 s 95ºC; 60 s 55ºC); For soybean Lec system: 120 s 50ºC; 10 min 95ºC; 45 x (15 s 95ºC; 60 s 60ºC)	7
For 40-3-2 system: 120 s 50ºC; 10 min 95ºC; 45 x (25 s 95ºC; 60 s 55ºC); For soybean Lec system: 120 s 50ºC; 600s 95ºC; 45 x (25 s 95ºC; 60 s 60ºC)	1

11.4. Standard ramp rate ?	**No. of laboratories**
a) Yes	74
b) No **of which**	6
Standard for Fast cycling on ABI7500 Fast	2
9600 emulation. Up: 0.8ºC/s, Down: 1.6ºC/s	3
15 min 95ºC ; 20ºC/s; 10 s 95ºC, 20ºC/s; 30 s 60ºC, 20ºC/s; 30 s 72ºC, 2ºC/s; 10 s 40ºC, 20ºC/s	1

12. Real-time PCR plate	**No. of laboratories**
a) 96-well plate	92
b) 384-well plate	1
c) **Other of which**	11
100-wells circle	1
48-well plate	1
72-tube rotor	1
72-well rotor	1
Capillaries	7

12.2 Reaction volume in µL	**No. of laboratories**
a) 20	19
b) 25	65
c) 50	15
d) **Other of which**	5
10	1
15	1
30	1
35	1
35	1

13. Real-time PCR mastermix	No. of laboratories
a) ABI TaqMan® Universal PCR master mix	48
b) ABI TaqMan® Universal PCR master mix, no AmpErase® UNG	8
b) ABI TaqMan® Universal PCR master mix, no AmpErase® UNG, v) Other : ABI TaqMan PCR core reagents kit	1
c) ABI TaqMan® Fast Universal PCR master mix	2
d) ABI TaqMan® Gold with Buffer A	3
e) Eurogentec: qPCR MasterMix	1
f) Sigma JumpstartTM Taq ReadyMix TM	1
h) Qiagen: QuantiTect Probe PCR kit	3
i) Roche: FastStart TaqMan® Probe Master (Rox)	1
k) Diagenode: Universal Mastermix	3
l) Eurofins: GMOQuant RoundUpReadyTM soy	7
o) Eurogentec qPCR MasterMix	1
p) Fermentas: Maxima™ Probe/ROX qPCR Master Mix	1
u) Proprietary real-time PCR master mix	1
v) **Other of which**	23
5Prime: RealMasterMix Probe 2.5x	1
ABI Taq, Buffer, MgCl2, but all separated.	1
ABI TaqMan 2xPCR Master Mix	1
ABI TaqMan Core Reagent Kit	2
Agilent Brilliant II QPCR Mastermix	1
Biotecon Diagnostics	1
Commercial RRS kit	1
Congen SureFood GMO Roundup Ready Soya Kit	1
Eurogentec: qPCR MasterMix Plus	1
In-house developed	1
Invitrogen	1
Invitrogen Platinum plus	1
Merck Light Cycler GMO Soya Quantification Kit	1
Metabion mi-Taq polymerase 1U; 1xbuffer supplied with polymerase; 3mM $MgCl_2$; 1xROX (Invitrogen); 400nM dNTP; primers and probes as in QT/GM/005	1
Roche Faststart DNA Master Hybprobe	2
Roche LightCycler 480 Probes Master	2
Roche Lightcycler 480 SYBR green I master	1
Roche LightCycler TaqMan Master	1
SureFood GMO Roundup Ready Soya Lec Reaction mix and Pet Reaction Mix from the kit	1
Taq Man GMO 35S Soy PCR Mix, Applied Biosystems	1

13.2. Number of reagents involved	No. of laboratories
a) 3	8
b) 4	16

c) 5	54
d) 6	7
e) **Other of which**	18
1	1
2	1
7	4
8	4
9	2
11	3
12	2
13	1

14.1. Sample intake (in ng) per real-time PCR reaction	**No. of laboratories**
0-100	42
100-200	39
200-300	6
300-400	0
400-500	4
> 500	1

Questions 14.2 to 14.5 only had to be answered in case of different sample intakes per real-time PCR

14.2. Sample intake (in ng) per real-time PCR reaction	**No. of laboratories**
0-100	19
100-200	8
200-300	1
300-400	1
400-500	1
> 500	1

14.3. Sample intake (in ng) per real-time PCR reaction	**No. of laboratories**
0-100	12
100-200	5
200-300	1
300-400	2
400-500	1
> 500	0

14.4. Sample intake (in ng) per real-time PCR reaction	**No. of laboratories**
0-100	5
100-200	5

200-300	1
300-400	2
400-500	1
> 500	0

14.5. Sample intake (in ng) per real-time PCR reaction	**No. of laboratories**
0-100	2
100-200	1
200-300	1
300-400	1
400-500	1
> 500	0

15.1. Sample intake (in µL) per real-time PCR reaction	**No. of laboratories**
< 1	1
1	6
2	15
3	2
4	8
5	45
6-10	8

Questions 15.2 to 15.5 only had to be answered in case of different sample intakes per real-time PCR

15.2. Sample intake (in µL) per real-time PCR reaction	**No. of laboratories**
1	2
2	1
3	0
4	1
5	3
6-10	1

15.3. Sample intake (in µL) per real-time PCR reaction	**No. of laboratories**
1	2
2	1
3	0
4	1
5	2
6-10	1

15.4. Sample intake (in µL) per real-time PCR reaction	**No. of laboratories**
1	2
2	1
3	0
4	1
5	0
6-10	1

15.5. Sample intake (in µL) per real-time PCR reaction	**No. of laboratories**
1	0
2	0
3	0
4	0
5	0
6-10	1

16. Number of reactions per DNA extraction	**No. of laboratories**
a) 1	2
b) 2	33
c) 3	31
d) 4	14
e) 5	1
f) 6	18
g) **Other of which**	4
8	1
9	1
10	1
12	1

17. Number of real-time PCR cycles	**No. of laboratories**
a) 40	12
c) 45	76
e) 50	15
f) **Other of which**	1
55	1

18. Real-time PCR detection method used?	**No. of laboratories**
a) MGB	6
b) Roche probe	1
c) Taqman probe	89
d) SYBRGreen	2
e) **Other of which**	9

TAMRA instead of MGBFQ	1
Taqman FAM-MGB and FAM-TAMRA	1
Taqman / MGB	1
MGB Probe for GM target, Taqman Probe for endogenous target	3
Lectin: TAMRA, GM target:MGBNFQ	1
Gel based	2

19. Real-time PCR quantification method used?	**No. of laboratories**
a) DNA copy number standard curve using a dilution series	37
b) Mass/mass standard curve using a dilution series	43
c) Delta Ct method	19
d) **Other of which**	5
Both a) and c)	1
Both b) and c)	1
Factor to convert to m/m %, this factor is calculated with two CRMs	1
Percentage (%) Standard Curve Using Dilution Series from one standard	1
Qualitative Gel based	1

20. For standard curve approach: slope - endogenous gene	**No. of laboratories**
a) -4.1 ≤ slope < -3.6	7
b) -3.6 ≤ slope ≤ -3.1	76
c) -3.1 < slope < -2.6	2
d) Other	0

21. For standard curve approach: slope – GM trait gene	**No. of laboratories**
a) -4.1 ≤ slope < -3.6	10
b) -3.6 ≤ slope ≤ -3.1	73
c) -3.1 < slope < -2.6	3
d) Other	0

22. For standard curve approach: R^2 coefficient - endogenous gene	**No. of laboratories**
a) 0.97 < R^2 < 0.98	5
b) 0.98 ≤ R^2 ≤ 0.99	15
c) 0.99 ≤ R^2 ≤ 1.00	64
d) Other	0

23. For standard curve approach: R^2 coefficient – GM trait gene	**No. of laboratories**
a) $0.97 < R^2 < 0.98$	3
b) $0.98 \leq R^2 \leq 0.99$	26
c) $0.99 \leq R^2 \leq 1.00$	55
d) Other	0

24. For standard curve approach: dynamic working range of the calibration curve - endogenous gene	**No. of laboratories**
100 - 0.16 ng/ react	1
100 - 100 ng	1
150 - 0.3 ng	1
200 - 50 - 12.5 - 4.17 - 1.39 ng/5 mL	1
200; 40; 8; 1.6; 0.32 ng	2
300 - 3.7 ng	1
40; 10; 2.5; 0.6; 0.15 ng	1
100000 - 40 cp	1
100000; 10000; 1000; 100 cp	1
101000 - 25 cp	1
125000; 30850; 7750; 1950 cp	1
132743; 44248; 14749; 4916; 1229 cp	1
141593; 70796; 35398; 17699; 8850 cp	1
157000 - 3950 cp	1
176991; 44248; 11062; 2765 cp	1
176991; 44248; 11062; 3687; 1229 cp	2
200000 - 16 cp	1
200000 - 20 cp	2
204800 - 1600 cp	1
204800; 51200; 12800; 3200; 800; 200 relative cp	2
216000; 72200; 24100; 8020; 2000 cp	1
221239 cp – 354 cp	2
250000 - 20 cp	3
265487 - 6555 cp	1
277655; 265487; 88496; 44248; 22124; 11062; 5531; 2765 cp	1
41000; 10250; 2563; 641; 160; 40 cp	1
50000 - 80 cp	1
50000; 10000; 2000; 400 cp	1
50000; 10000; 2000; 500; 100 cp	1
51250, 10250, 2050, 410, 82, 16 cp	1
60000; 6000; 600; 60; 30 cp	1
79431 - 20 cp	1
80000; 16000; 8000; 2000 cp	1

81920 - 160 cp	2
81920; 10240; 1280; 160 copies	3
86100; 28700; 9567; 3189 cp	1
86400; 14400; 2400; 400 cp	1
86960 - 1400 cp	1
88500; 17700; 3540; 708 cp	1

25. For standard curve approach: dynamic working range of the calibration curve - GM trait gene	**No. of laboratories**
18.75 - 0.07 ng	1
20; 4; 0.8; 0.16; 0.032 ng	2
20; 5; 1.25; 0.42; 0.14 ng/5 mL	1
30 - 0.37 ng	1
4; 1; 0.25; 0.06; 0.015 ng	2
5 - 0.008 ng/react	1
5 - 0.05 ng	1
10000;1000; 100; 50 cp	1
10240 - 40 cp	2
10240; 1280; 160; 40 cp	3
10240; 2560; 640; 160; 40 relative cp	2
13274 - 327 cp	1
14159; 7080; 3540; 1770; 885 cp	1
17699; 4425; 1106; 277 cp	1
17699; 4425; 1106; 369; 123 cp	2
17699; 8850; 4425; 2212; 1106; 553; 276; 80 cp	1
18200 - 4 cp	1
1986 - 20 cp	1
200000 - 16 cp	1
200000 - 20 cp	2
20480 - 40 cp	1
22124 - 35 cp	2
24100; 8020; 2000; 501, 167 cp	1
2500; 617; 155; 39 cp	1
250000 - 20 cp	1
250000; 20000; 1500; 125; 20 cp	2
2560 - 40 cp	1
4100; 1025; 256; 64; 16; 4 cp	1
4305; 1435; 478; 159 cp	1
4425; 885; 177; 35 cp	1
5000 - 10 cp	1
5000; 1000; 200; 40; 20 cp	1
5000; 1000; 200; 50; 10 cp	1

51250; 10250; 2050; 410; 82; 16 cp	1
5400; 540; 270; 90; 45 cp	1
6637; 2212; 737; 246; 61 cp	1
7900 - 200 cp	1
800; 200; 50; 20 cp	1
8640; 1440; 240; 40 cp	1
8696 - 140 cp	1

26. For Delta Ct method: slope	**No. of laboratories**
a) -4.1 ≤ slope < -3.6	3
b) -3.6 ≤ slope ≤ -3.1	39
c) -3.1 < slope < -2.6	5
d) Other	56

27. For Delta Ct method: dynamic working range of the calibration curve	**No. of laboratories**
10 - 0,1 ng	1
10 % - 0.1 % to m/m CRMs	1
10; 5; 4; 3; 2; 1; 0 ng	1
250000; 20000; 1500; 125; 20; 0 cp	1

28. For Delta Ct method: R^2 coefficient	**No. of laboratories**
a) 0.97 < R^2 < 0.98	3
b) 0.98 ≤ R^2 ≤ 0.99	15
c) 0.99 ≤ R^2 ≤ 1.00	27
d) **Other of which**	1
0.96	1

29. Endogenous target DNA sequence for RoundUp Ready soybean?	**No. of laboratories**
a) Lectin	103
b) **Other of which**	1
Absolute quantification, endogenous control was not used	1

30. Amplicon size (in bp) – endogenous gene	**No. of laboratories**
29	1
63	2
74	57

76	2
79	1
80	1
81	14
83	1
88	1
100	1
102	2
105	1
112	1
118	7
120	2
123	1
145	1
181	1
318	1

31. Primer and probe sequences – endogenous gene

31.1 F-primer	No. of laboratories
AAC CGG TAG CGT TGC CAG	4
CAC CTT TCT CGC ACC AAT TGA CA	1
CCA GCT TCG CCG CTT CCT TC	47
CCA GCT TCG TCG CCG CTT CCT TC	1
CCG GAA AGG CCA GAG GAT	1
CGG CAC CCC AAA ACC C	1
CTT TCT CGC ACC AAT TGA CA	2
GAC GCT ATT GTG ACC TCC TC	3
GAT AGT GGG ATT CGT CA	1
GCC CTC TAC TCC ACC CCC A	9
GCC CTC TAC TCC ACC CCC ATC C	3
TCC ACC CCC ATC CAC ATT T	14
TCT CCG ATG TGG TCG ATT TG	1
TGG TCG CGC CCT CTA CTC	2
In-house developed	1
Unknown, proprietary	8

31.2 R-primer	No. of laboratories
ACG TCA TGC GAT TCC CCA GG	1
AGC CCA TCT GCA AGC CTT T	4
GAA AGT GTC AAG CTT AAC AGC GAC G	1
GAA GGC AAG CCC ATC TCG AAG CC	48
GCC CAT CTG CAA GCC TTT TT	9
GCC CAT CTG CAA GCC TTT TTG TG	3
GCT ACC GGT TTC TTT GTC CCA	1
GGA TTT CAG CAT CAG TGG CTA CA	1
GGC ATA GAA GGT GAA GTT GAA GGA	14

GGC GAA GCT GGC AAC G	2
TCA AAC TCA ACA GCG ACG AC	3
TGT CAG GGG CAT AGA AGG TG	2
In-house developed	1
Unknown, proprietary	8

31.3 Probe	**No. of laboratories**
AAC CGG TAG CGT TGC CAG CTT CG	4
AGC TTC GCC GCT TCC TTC AAC TTC AC	4
CTA CCG GTT TCT TTG TCC CAA ATG TGG AT	2
CTT CAC CTT CTA TGC CCC TGA CAC	10
FAM-AAC CGG TAG CGT TGC CAG CTT CG-TAMRA	6
FAM-AGC TCC GCC GCT TCC TTC AAC TTC AC-TAMRA	1
FAM-AGC TTC GCC GCT TCC TTC AAC TTC AC-TAMRA	4
FAM-CAA CTC AAT AAG GTT GAC GAA AAC GGC-TAMRA	2
FAM-CCA CAA ACA CAT GCA GGT TAT CTT GG-TAMRA	2
FAM-CCA CAA ACA CAT GCA GGT TAT CTT GGT-TAMRA	1
FAM-CTC TTG GTC GCG CCC TCT ACT CCA C-TAMRA	1
FAM-CTT CAC CTT CTA TCG CCC TGA CAC-TAMRA	1
FAM-CTT CAC CTT CTA TGC CCC TGA CAC-BHQ	1
FAM-CTT CAC CTT CTA TGC CCC TGA CAC-TAMRA	33
FAM-TTC GCC GCT TCC TTC AAC TTC ACC T-TAMRA	1
HEX-CTA CCG GTT TCT TTG TCC CAA ATG TGG AT-TAMRA	1
HEX-CTT CAC CTT CTA TGC CCC TGA CAC-TAMRA	1
TTC CCG AGT GGG TGA GGA TA	1
TTC GCC GCT TCC TTC AAC TTC ACC T	2
VIC-AAC CGG TAG CGT TGC CAG CTT CG-TAMRA	5
VIC-TTC GCC GCT TCC TTC AAC TTC ACC T-TAMRA	1
Yakima Yellow-CTT CAC CTT CTA TGC CCC TGA CAC-TAMRA	1
YY-CTT CAC CTT CTA TGC CCC TGA CAC-BHQ1	1
In-house developed	1
Unknown, proprietary	9

32. GM trait target DNA sequence for 40-3-2 soybean?	**No. of laboratories**
a) 35S promoter	7
b) CTP4 – chloroplast targeting sequence	1
c) CP4 EPSPS	9
d) RoundUp Ready soybean-specific	66

e) *Nos* terminator	1
f) **Other of which**	18
35S promoter - chloroplast targeting sequence	1
35S promoter - CTP1 chloroplast targeting sequence	1
35S promotor-CP4 EPSPS	2
CTP 35S	1
CTP4-EPSPS junction (construct-specific)	2
DNA sequence in the 5' IBR	1
EPSPS	1
Junction CTP with CP4 (RRS construct-specific)	1
Junction region between the Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and the chloroplast transit peptide (CTP) sequence from *Petunia hybrida epsps* gene	7
Ready to use primer and Hybridization Probe mix, specific for the 35S promoter and CTP4	1

33. Amplicon size (in bp) – GM trait gene	**No. of laboratories**
21	1
62	2
74	13
81	2
83	12
84	35
85	11
89	1
94	2
101	1
105	1
110	1
118	1
121	7
123	1
127	1
128	1
132	1
140	1
147	1
172	2
195	1

34. Primer and probe sequences – GM trait gene

34.1. F-primer	**No. of laboratories**
ATG CAG GTC CAT CGG CA	1
ATT GAT GTG ATA TCT CCA CTG ACG T	1

CAT TCC CGG CGA CAA GTC	1
CAT TTG GAG AGG ACA CGC TGA	13
CCG GAA AGG CCA GAG GAT	3
CCT TTA GGA TTT CAG CAT CAG TGG	10
CGC AAT GAT GGC ATT TGT AGG	2
CTC GAT TTC GGC AAT GCC GC	1
CTT GCC CGT ATT GAT GAC GTC	1
GAT AGT GGG ATT GTG CGT CA	1
GCA AAT CCT CTG GCC TTT CC	1
GCC ATG TTG TTA ATT TGT GCC AT	1
GCC ATG TTG TTA ATT TGT GCC AT	13
GCT CCT ACA AAT GCC ATC A	1
TAG CAT CTA CAT ATA GCT TC	5
TGA TGT GAT ATC TCC ACT GAC G	2
TTC ATT CAA AAT AAG ATC ATA CAT ACA GGT T	33
TTC ATT CAAG ATC ATA CAT ACA GGT T	1
In-house developed	1
Unknown, proprietary	8

34.2. R-primer	**No. of laboratories**
ATG CAG GTC CAT CGG CA	1
CAG CAG AGA TCC CCA GGA AG	1
CCT CTC CAA ATG AAA TGA ACT TCC T	1
CTT GCC CGT ATT GAT GAC GTC	1
GAA GTT CAT TTC ATT TGG AGA GGA C	14
GAC CAG GCC ATT CGC CTC A	5
GAC TTG TCG CCG GGA ATG	10
GAG CCA TGT TGT TAA TTT GTG CC	13
GAT AGT GGG ATT GTG CGT CA	1
GCA AAT CCT CTG GCC TTT CC	1
GCT CCT ACA AAT GCC ATC A	1
GGA TTT CAG CAT CAG TGG CTA CA	3
GGC ATT TGT AGG AGC CAC CTT	34
TGT ATC CCT TGA GCC ATG TTG T	2
TTG ATG ACG TCC TCG CCT TC	1
TTT CAT TCA AAA TAA GAT CAT ACA TAC AGG TTA	2
In-house developed	1
Unknown, proprietary	8

34.3. Probe	**No. of laboratories**
ACA AAA CTA TTT GGG ATC GGA GAA GA	2
CAA GCT GAC TCT AGC AGA TCT TTC	6
CCG GCT GCT TGC ACC GTG AAG	2
CCT TTT CCA TTT GGG	2
CGA TTT CAA GCG CAT CAT GCT GGG	1
CGC AAC CGC CCG CAA ATC C	4

FAM -CCT TTT CCA TTT GGG-TAMRA	1
FAM-ACA AAA CTA TTT GGG ATC GGA GAA GA-TAMRA	3
FAM-ACC TTC CTT TTC CAT TTG GGT TCC CTA TGT TTA TTT-TAMRA	2
FAM-ATG CAG GTC CAT CGG CA-TAMRA	1
FAM-CAA GCT GAC TCT AGC AGA TCT TTC-TAMRA	6
FAM-CCA GCT GAC TCT AGC AGA TCT TTC- TAMRA	1
FAM-CCC ACT ATC CTT CGC AAG ACC CT-TAMRA	2
FAM-CCC ACT ATC CTT CGC AAG ACC CTT CCT-TAMRA	1
FAM-CCG GCT GCT TGC ACC GTG AAG-TAMRA	1
FAM-CCT TCA TGT TCG GCG GTC TCG C-TAMRA	1
FAM-CCT TTT + CCAT + T + T + GGG-TAMRA (+=LNA-base)	1
FAM-CCT TTT CCA TTT GGG	1
FAM-CCT TTT CCA TTT GGG-MGB	3
FAM-CCT TTT CCA TTT GGG-MGBNFQ	22
FAM-CCT TTT CCA TTT GGG-TAMRA	3
FAM-CGC AAC CGC CCG CAA ATC C-TAMRA	6
FAM-CTT GAA AGA TCT GCT AGA GTC AGC TTG TCA GCG-TAMRA	10
FAM-TTC ATG TTC GGC GGT CTC GCG-TAMRA	1
TTC ATG TTC GGC GGT CTC GCG	1
In-house developed	1
Unknown, proprietary	8

35. Which reference material was used for calibration?	**No. of laboratories**
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series	10
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series, c) ERM-BF410a	2
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series, h) ERM-BF410dk, j) ERM-BF410gk	1
b) ERM-BF410bk, ERM-BF410dk, ERM-BF410gk series	4
b) ERM-BF410bk, ERM-BF410dk, ERM-BF410gk series, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk	1
b) ERM-BF410bk, ERM-BF410dk, ERM-BF410gk series, g) ERM-BF410c	1
c) ERM-BF410a	1
c) ERM-BF410a, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, i) ERM-BF410e	1
c) ERM-BF410a, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, i) ERM-BF410e, j) ERM-BF410gk	1
c) ERM-BF410a, e) ERM-BF410b, g) ERM-BF410c,	1

i) ERM-BF410e, j) ERM-BF410gk	
d) ERM-BF410ak, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk, ERM-BF410f, ERM-BF410e	1
e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, i) ERM-BF410e, j) ERM-BF410gk	1
e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk	3
e) ERM-BF410b, h) ERM-BF410dk, j) ERM-BF410gk	1
f) ERM-BF410bk, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk	1
h) ERM-BF410dk, j) ERM-BF410gk, ERM-BF 410f	1
i) ERM-BF410e	3
j) ERM-BF410gk	34
k) Eurofins GeneScan reference material	8
k) Eurofins GeneScan reference material, Home-made 18 % calibrated from 5 % CRM	1
l) **Other of which**	37
100% RoundUp Ready leaf material	1
ABI Calibration kit	1
Calibrator DNA contains a stabilised solution of plasmid DNA	1
Congen SureFood GMO Roundup Ready Soya Kit	2
Conventional Soybean from National reference lab	1
CRM-IRMM410	1
ERM-BF 410f	12
ERM-BF410d, ERM-BF410f	1
ERM-BF410f (GM) ERM-BF410ak (lectin)	1
gDNA	4
GM Soybean (RRS) Detection Plasmid set (Nippon Gene/Diagenode)	4
Home-made 18 % calibrated from 5% CRM	1
In-house seeds 100 % RRS	1
IRMM 410R	1
IRMM-410S-5	1
Multi Target Plasmids, Nippon Gene	1
pDNA	3

36. Which reference material was used for quality control?	**No. of laboratories**
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series	9
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series, c) ERM-BF410a	2
a) ERM-BF410b, ERM-BF410c, ERM-BF410e series, l) Other	1
b) ERM-BF410bk, ERM-BF410dk, ERM-BF410gk series	7

b) ERM-BF410bk, ERM-BF410dk, ERM-BF410gk series, g) ERM-BF410c	1
c) ERM-BF410a	3
c) ERM-BF410a, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, i) ERM-BF410e, j) ERM-BF410gk	2
c) ERM-BF410a, e) ERM-BF410b, h) ERM-BF410dk	1
c) ERM-BF410a, g) ERM-BF410c	1
c) ERM-BF410a, j) ERM-BF410gk	1
d) ERM-BF410ak, e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk	2
e) ERM-BF410b	3
e) ERM-BF410b, g) ERM-BF410c	1
e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk	2
e) ERM-BF410b, g) ERM-BF410c, h) ERM-BF410dk, j) ERM-BF410gk	1
e) ERM-BF410b, h) ERM-BF410dk	6
e) ERM-BF410b, h) ERM-BF410dk, j) ERM-BF410gk	1
e) ERM-BF410b, i) ERM-BF410e	1
e) ERM-BF410b, l) Other	3
f) ERM-BF410bk	1
g) ERM-BF410c	4
g) ERM-BF410c, h) ERM-BF410dk, i) ERM-BF410e, l) Other	1
h) ERM-BF410dk	12
h) ERM-BF410dk, i) ERM-BF410e, l) Other	1
h) ERM-BF410dk, j) ERM-BF410gk	1
i) ERM-BF410e	1
j) ERM-BF410gk	5
j) ERM-BF410gk, l) Other	1
k) Eurofins GeneScan reference material	6
l) **Other of which**	29
2,5 % MON-04032-6	1
A sample from GIPSA interlaboratory comparison	1
A sample of GeMMA proficiency testing	2
ERM-BF410b, ERM-BF410f	1
ERM-BF410d	10
ERM-BF410d, ERM-BF410a	1
ERM-BF410d, ERM-BF410f	3
ERM-BF410f	4
ERM-BF410f (GM), ERM-BF410a (lectin)	1
EU Ref material blank and kits control sample	1
In-house control	1
Negative control (H_2O), extraction control, ERM-BF410	1
Previous proficiency test material for RoundUp Ready soya	1

Water and conventional wheat	1

37. Practical LOD (in %) of the GM content determination for GM level 1 and level 2
38. Practical LOQ (in %) of the GM content determination for GM level 1 and level 2

The answers to questions 37 and 38 are shown in Tables 2 to 5.

39. Did you report uncertainty as an absolute value?	**No. of laboratories**
a) Yes	55
b) No	48

39.1. If you have responded yes to 39, does the uncertainty correspond to a repeatability standard deviation?	**No. of laboratories**
a) Yes	33
b) No	19
c) Not applicable	16

39.2. If you have responded no to 39.1, does the uncertainty correspond to a within-laboratory reproducibility?	**No. of laboratories**
a) Yes	28
b) No	7
c) Not applicable	27

39.3. Does the uncertainty include a contribution from the heterogeneity of the material?	**No. of laboratories**
a) Yes	10
b) No	40
c) Not applicable	29

39.4 If you have responded yes to 39.3, please specify the equation you used for the calculation of the uncertainty	**No. of laboratories**
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories, EURL + IRMM, 2009, equation 12 and 14	1
Horwitz	1
JRC Scientific and Technical Reports: EUR 22756 EN/2-2009	1

39.5 If the approach for the estimation of the uncertainty is different from what is described above, please give the equation used for the calculation of the uncertainty	**No. of laboratories**
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories, EU-RL + IRMM, 2009, equation 12	1
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories, EU-RL + IRMM, 2009, equation 12 and 14	1
JRC Scientific and Technical Reports, EUR22756 EN/2, ISBN 978-92-7911228-7	1
Not able to clearly establish the value	1
Holistic method	1

39.6. Did you report an expanded uncertainty including a coverage factor?	**No. of laboratories**
a) Yes	53
b) No	15
c) Not applicable	14

39.7. If you have responded yes to 39.6, please specify the coverage factor used (k = 1 for a 66.67 % confidence level, k = 2 for a 95 % confidence level, k = 3 for a 99 % confidence level).	**No. of laboratories**
b) k = 2	56

40. Did you report the uncertainty as a relative value (i.e. in %)?	**No. of laboratories**
a) Yes	40
b) No	53

40.1. If you have responded yes to 40, does the value reported correspond to a percentage of the GM level reported?	**No. of laboratories**
a) Yes	29
b) No	10
c) Not applicable	17

40.2. Does the uncertainty correspond to a relative repeatability standard deviation?	**No. of laboratories**
a) Yes	22
b) No	15
c) Not applicable	21

40.3. If you have responded no to 40.2, does the uncertainty correspond to a relative within-laboratory reproducibility?	No. of laboratories
a) Yes	9
b) No	10
c) Not applicable	24

40.4. Does the uncertainty include a contribution from the heterogeneity of the material?	No. of laboratories
a) Yes	13
b) No	40

40.5 If you have responded yes to 40.4, please specify the equation you used for the calculation of the uncertainty	No. of laboratories
Guidance Document on Measurement Uncertainty for GMO Testing Laboratories, EURL + IRMM, 2009, equation 12 and 14	1
Scholtens, I.M.J., Kok, E.J., Hougs, L., Molenaar, B., Thissen, J.T.N.M., and H. van der Voet. 'Increased efficacy for in-house validation of real-time PCR GMO detection methods.' Anal. Bioanal. Chem. (2010), 6, 2213-2227	1

40.6 If the approach for the estimation of the uncertainty is different from what is described above, please give the equation used for the calculation of the uncertainty	No. of laboratories
Scholtens, I.M.J., Kok, E.J., Hougs, L., Molenaar, B., Thissen, J.T.N.M., and H. van der Voet. 'Increased efficacy for in-house validation of real-time PCR GMO detection methods.' Anal. Bioanal. Chem. (2010), 6, 2213-2227	1

40.7. Did you report an expanded uncertainty including a coverage factor?	No. of laboratories
a) Yes	31
b) No	12
c) Not applicable	12

40.8. If you have responded yes to 40.7, please specify the coverage factor used (k = 1 for a 66.67 % confidence level, k = 2 for a 95 % confidence level, k = 3 for a 99 % confidence level).	No. of laboratories
b) k = 2	31

12. Acknowledgements

With respect to the raw material used in this study we kindly acknowledge Monsanto Company Corporation for providing the soybean event 40-3-2. We sincerely thank Roberta Brustio, Maddalena Chessa, Stéphane Cordeil, Barbara Munaro, Steven Price, Eleonora Scigliano, Pierluigi Tenuta, Stefania Tomasina of the MBG Unit and EU-RL GMFF for their invaluable contributions to this third comparative testing round. A special thanks to Marko Maras who is very actively involved in the comparative testing activities. We acknowledge Fernando Cordeiro Raposo, Beatriz De la Calle, Franz Ulberth, Inge Verbist from the FSQ Unit of IRMM for the on-line registration of participants and the management of the reported results.

The labs listed below are kindly acknowledged for their participation.

Organisation	Department	Country	Status
A BioTech Lab	Laboratory for Biotechnology	RS	4
Agenzia provinciale per l'ambiente	Laboratorio analisi alimenti	IT	5
Austrian Agency for Health and Food Safety (AGES)	Competence Centre Biochemistry	AT	1, 2
Agricultural Institute of Slovenia		SI	2
Agri-Food and Veterinary Authority of Singapore	Laboratory Department	SG	4
Agroscope Liebefeld-Posieux Research Station ALP	Analytics	CH	4
American University of Science & Technology		LB	4
Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)	Laboratoire de la Santé des Végétaux	FR	1, 2
ARPA Piemonte	Polo Alimenti	IT	5
Bavarian Health and Food Safety Authority		DE	2
Biomi Ltd.		HU	3
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit		DE	1
Central Agricultural Office, Food and Feed Safety Directorate	Laboratory for GMO Food	HU	1, 2
Central Agricultural Office, Food and Feed Safety Directorate	Feed Investigation NRL	HU	1, 2
Central Control and Testing Institute of Agriculture	Molecular Biology	SK	1, 2
Centro Nacional de Alimentación (Agencia Espaňola de seguridad alimentaria y nutricion)	Biotechnology Unit	ES	1, 2
Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL)		DE	3
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)		DE	2
Consorcio CSIC-IRTA-UAB	SABQ	ES	3
CRA-W (Centre wallon de Recherches agronomiques)	Valorization of Agric. Prod.	BE	1, 2
Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Seed and Seedlings	Seed Testing Laboratory	HR	3
Croatian National Institute of Public Health	GMO Quant. and RA Unit	HR	4
Crop Research Institute	Molecular Biology RLGMO	CZ	1, 2
Danish Veterinary and Food Administration	Division of Plant Diagnostics	DK	1, 2
DTU-Food, National Food Institute	Toxicology and Risk Assessment	DK	1, 2
Executive Environmental Agency		BG	3
Federal Institute for Risk Assessment (BfR)	Effect-based Analytics and Tox	DE	2
Federal Office of Public Health FOPH	Consumer Protection Directorat	CH	3
Food and Environment Research Agency (FERA)*		IE	1
Food and Environment Research Agency (FERA)*		UK	2
Finnish Customs Laboratory	ET2 / BIO	FI	1, 2
Food and consumer product safety authority	Laboratory	NL	2
Groupe d'Etude et de contrôle des Variétés et des Semences (GEVES)	BioGEVES	FR	1, 2
Hessisches Landeslabor		DE	2
INRAN - Seed Testing Station	Laboratorio Analisi Sementi	IT	2
Institute for Agricultural and Fisheries Research (ILVO)	Technology and Food Sciences	BE	1, 2
Institut für Hygiene und Umwelt	Gentechnik	DE	2
Institute for Diagnosis and Animal Health	Molecular Biology and GMO Unit	RO	1
Institute for Genetic Engineering and Biotechnology		BA	4
Institute of Biochemistry and Biophysics PAS		PL	2
Institute of Food Safety, Animal Health and Environment „BIOR"	Virology department	LV	1, 2
Instituto Nacional de Recursos Biológicos (INRB)	Laboratório de Caracterização de Materiais de Multiplicaçâo de Plantas	PT	2
Instytut Zootechniki Państwowy Instytut Badawczy	Krajowe Laboratorium Pasz Prac	PL	1, 2

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"	Reparto Igiene degli Alimenti	IT	5
Istituto Superiore di Sanità - National Institute of Health	DSPVSA GMO and Mycotoxins Unit	IT	2
Istituto Zooprofilattico delle Venezie	SC1-Microbiologia Alimentare	IT	5
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta	S.C. Biotecnologie	IT	5
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna	Reparto Genomica	IT	5
Istituto Zooprofilattico Sperimentale della Sardegna	Igiene alimenti	IT	5
Istituto Zooprofilattico Sperimentale Lazio e Toscana	Biotecnologie	IT	1, 2
Istituto Zooprofilattico Sperimentale Umbria e Marche	Laboratorio OGM	IT	5
Kenya Agricultural Research Institute	Kari-Njoro	KE	4
Kenya Plant Health Inspectorate Service	Phytosanitary Department, Molecular Biology Laboratory	KE	4
Kyung Hee University		KR	4
Laboratoire national de santé	Food control	LU	1, 2
Laboratorio Arbitral Agroalimentario - MARM	OGM	ES	1, 2
Landesamt für Umweltschutz Sachsen-Anhalt	FG13	DE	2
Landesamt für Verbraucherschutz Sachsen-Anhalt	Fachbereich 3	DE	2
Landeslabor Berlin Brandenburg	Fb. I-6	DE	2
Landeslabor Schleswig-Holstein		DE	2
Landesuntersuchungsamt Rheinland-Pfalz	Institut f. Lebensmittelchemie	DE	2
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen (LUA)	Amtliche Lebensmitteluntersuch	DE	2
Lower Saxony Federal State Office for Consumer Protection and Food Safety (LAVES)	State Food Laboratory Braunschweig	DE	2
LGC Limited	Molecular and Cell Biology	UK	1, 2
LGV-Landesamt f. Gesundheit u. Verbraucherschutz	D5	DE	2
LTZ Augustenberg		DE	2
Ministério da Agricultura Pecuária e Abastecimento	LANAGRO-MG	BR	4
Ministry of Agriculture and Rural Afairs Ankara Provincial Control Laboratory	GMO Lab	TR	4
Ministry of Finance, General Secretariat for Tax and Customs Issues, General Chemical State Laboratory (GCSL)	Food Division Athens	GR	1, 2
National Bureau of Plant Genetic Resources	NRC on DNA Fingerprinting	IN	4
National Center of Public Health Protection	Laboratory for GM Food analyses	BG	1, 2
National Food Agency	Science Department	SE	1, 2
National Food and Veterinary Risk Assessment Institute	Molecular Biology and GMO Section	LT	1, 2
National Food Reference Laboratory	Biotechnology and GMO	TR	4
National Institute for Food and Drug Surveillance - INVIMA		CO	4
National Institute of Biology	Department of Biotechnology	SI	1, 2
National Institute of Public Health in Prague	Food Safety and Nutrition	CZ	2
National Public Health Laboratory, Ministry of Health	Food department	MY	4
National Research and Development Institute for Biotechnology in Horticulture	Research	RO	4
National Veterinary Institute	Food Bacteriology and GMO	NO	3
National Veterinary Research Institute	Feed Hygiene	PL	1, 2
Plant Breeding and Acclimatization Institute – National Research Institute	GMO Controlling Laboratory	PL	2
Regional Laboratory of Genetically Modified Food		PL	1, 2
RIKILT -Institute of Food Safety, WUR	NFA	NL	1, 2
RZI	SM	BG	4
Scientific Institute of Public Health	Platform Biotech & Mol Biol	BE	1, 2
Service Commun des Laboratoires du MINEFI - Laboratoire de Strasbourg		FR	1, 2

Servicio Agricola y Ganadero	De laboratorios y estaciones c	CL	4
Somerset County Council	Somerset Scientific Services	UK	3
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Geschäftsbereich 6	DE	2
Staatliches Veterinäruntersuchungsamt Arnsberg		DE	3
State Institute of Chemical and Veterinarian Analysis - Freiburg	Gentechnik	DE	2
State Office for Agriculture, Food Safety and Fishery Mecklenburg-Western Pomerania	Molecular Diagnostics	DE	2
State Veterinary and Food Institute Dolny Kubin	Dept. of mol. biol. analysis	SK	1, 2
Tallinn University of Technology	Gene Technology	EE	2
Thüringer Landesanstalt für Landwirtschaft	Untersuchungswesen	DE	3
Thüringer Landesamt für Verbraucherschutz und Lebensmittelsicherheit (TLLV)	Lab for detection of GMO/foods	DE	2
Ukrmetrteststandard	Molecular biology depaertment	UA	4
Umweltbundesamt		AT	1, 2
University of the Free State	GMO Testing Facility	ZA	4
USDA, GIPSA, FGIS	Biotechnology	US	4
Worcestershire Scientific Services		UK	3

1 Laboratory appointed under Regulation (EC) No 882/2004,
2 Laboratory appointed under Regulation (EC) No 1981/2006,
3 ENGL only member,
4 Laboratory from third country,
5 Official control laboratory

13. Annex 1: Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

EURL
European Union Reference Laboratory
for GM Food & Feed

Ispra, 23 February 2011
JRC.DG.I.4-MBG/GVdE/mc/ARES(2011)207560

NOTE FOR THE ATTENTION OF

I. **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.**

II. **All National Reference Laboratories nominated under COMMISSION REGULATION (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the European Union reference laboratory for genetically modified organisms.**

III. **All members of the European Network of GMO Laboratories**

IV. **Interested parties from third countries**

Subject: Invitation to participate in the comparative test ILC-EURL-GMFF-CT-01/11

Pursuing Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) has the obligation to organise comparative testing rounds and ensure an appropriate follow-up of the results obtained.

Hereby, I would like to invite you to participate in the third round of comparative testing ILC-EURL-GMFF-CT-01/11. This round of comparative testing will include two test materials of RoundUp Ready™ soybean (soybean line 40-3-2). The participant will need to quantify the GM level in each test material.

I would like to remind you that participation in comparative testing is mandatory for all National Reference Laboratories nominated under Regulation (EC) No 882/2004 and Regulation (EC) No 1981/2006. Your participation is free of charge.

Comparative testing is organised by the EURL-GMFF in collaboration with the Institute for Reference Materials and Measurements (IRMM, Geel, BE). Registration for the third round of comparative testing and submission of results will be handled by IRMM. Please register electronically for the third comparative testing round using the following link: https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=620

Joint Research Centre · 21027 Ispra VA, Italy · TP 331
Secretariat · Phone: +39 0332 789379, Fax: +39 0332 785483
E-mail: JRC-BGMO@ec.europa.eu
WWW: http://gmo-crl.jrc.ec.europa.eu/ · http://bgmo.jrc.ec.europa.eu · http://ihcp.jrc.ec.europa.eu

ISO 9001:2008 certified by
CERMET SINCERT

Please be aware that you need to submit multiple registration forms when you wish to apply different approaches of quantification (i.e. standard curve method, delta Ct method,…) or use different units of measurement for reporting your results.
Once you have submitted your registration electronically, print your registration form, sign it and send it to IRMM by fax or E-mail:

Fax: +32 14 571 865
Mail: JRC-IRMM-IMEP@ec.europa.eu
Cc to: mbg-comparative-testing@jrc.ec.europa.eu

Your fax/E-mail is the confirmation of your participation.

The deadline for registration is **8 March 2011**. Samples should be shipped during the week of **4 to 8 April 2011**. The deadline for submission of results is **20 May 2011**.

If you should have any questions related to the third round of comparative testing, please contact:

Diana Charels
European Commission – Joint Research Centre
Molecular Biology and Genomics Unit – TP201
Via E. Fermi 2749
I-21027 Ispra (VA)
Phone: +39 0332 78 6518
Fax: +39 0332 78 9333
E-mail: mbg-comparative-testing@jrc.ec.europa.eu

The EURL-GMFF is looking forward to your participation.

Yours sincerely,

Guy Van den Eede
Head of Molecular Biology and Genomics Unit

Copy: M. Mazzara, D. Charels, M. Maras, T. Weber, F. Ulberth (JRC).

14. Annex 2: Accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

Ispra, 01 April 2011
JRCI04/MBG/GVDE/st/ Ares(2011)360704

NOTE FOR THE ATTENTION OF

«AddressBlock»

«Zip»«Town»

«Country»

Subject: **Participation in ILC-EURL-GMFF-CT-01/11, a comparative testing round to quantify the GM content of RoundUp Ready™ soybean (soybean line 40-3-2) test items.**

Dear «Firstname» «Surname»,

Thank you for participating in the ILC-EURL-GMFF-CT-01/11 comparative testing round to quantify the GM content of RoundUp Ready™ soybean test items.

You will receive the test items shipped at room temperature via courier. The shipment will be carried out in the week of **4 to 8 April 2011**. On the day of the shipment we will inform you, by E-mail, about the parcel tracking number. Please make sure that someone in your laboratory is available to receive the parcel.

The parcel contains:

1. Two plastic containers each containing approximately 5 g of test item
2. An "Acknowledgement of Reception" form
3. This accompanying letter

Please check whether the plastic containers containing the test item remained undamaged during transport and return the "Acknowledgement of Reception" form by fax (+39 0332 789333). You should store the samples in a dark and cold place (not exceeding 18 ºC).

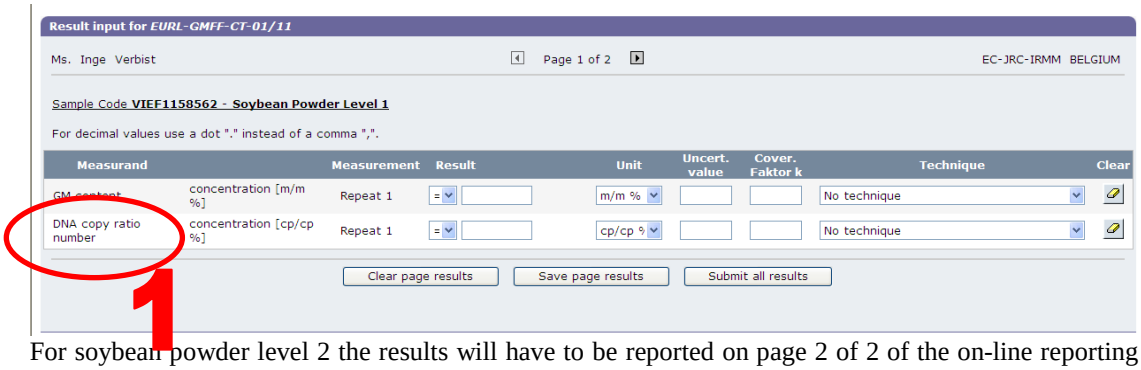
You should determine the GM level of RoundUp Ready™ soybean in each test item received. The procedure used for quantification should resemble as closely as possible the one that you use in routine sample analyses.

The results can be reported in mass/mass % and/or copy/copy % as outlined below:

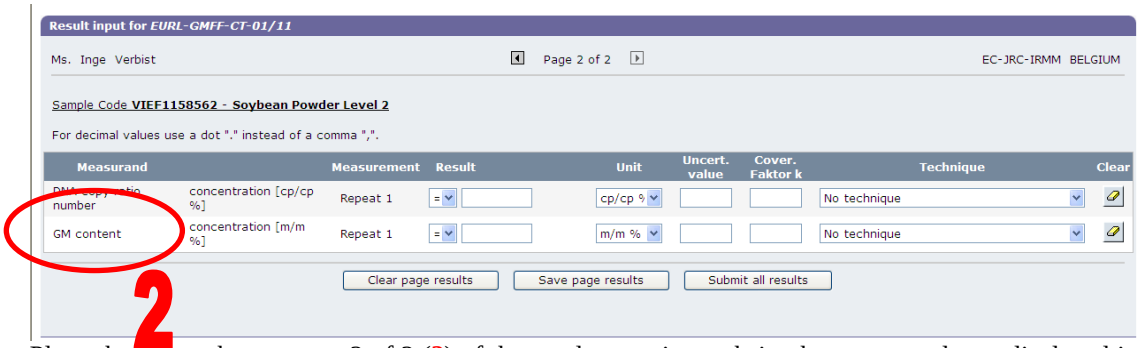
$$\text{mass/mass \%} = \frac{\text{mass GM [g]}}{\text{Total mass [g]}} \times 100\ \%$$

$$\text{copy/copy \%} = \frac{\text{GM DNA copy numbers [cp]}}{\text{Target taxon-specific DNA copy numbers [cp]}} \times 100\ \%$$

You can find the reporting website at https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do. You need a personal password to access this webpage which is «Part_key». The system will guide you through the reporting procedure. Please enter for each test item the measurement result with its associated uncertainty. For soybean powder level 1 the results will have to be reported on page 1 of 2 of the on-line reporting system. Please report your results either in GM content or DNA copy number ratio. The term technique displayed in the reporting form below refers to Real-time PCR quantification. Please select the option 'Not applicable' in the on-line reporting system for Technique.

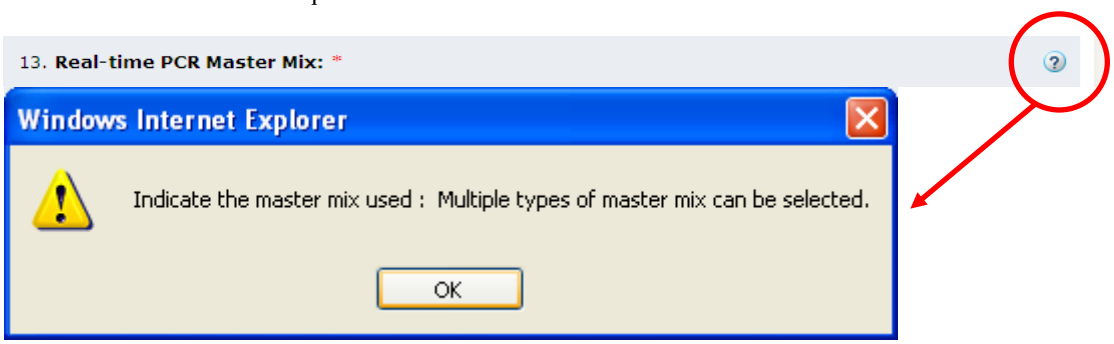


For soybean powder level 2 the results will have to be reported on page 2 of 2 of the on-line reporting system.



Please be aware that on page 2 of 2 (2) of the result reporting website the measurands are displayed in **another order** than on page 1 of 2 (1), therefore pay attention to report the results in the right place.

After entering all results, please complete the questionnaire. Items bearing a question mark icon on the right-hand side, as shown in the example below, contain additional information for the participant. In the reporting website clicking on the icon will give access to this information. Do not forget to save, submit and confirm when required to do so.



The pdf file of the questionnaire that you will or have already received by E-mail is intended as an aid in the laboratory. In this pdf file, items with the word '(number)' indicate that a numerical value should be provided. Pdf files of questionnaires bearing hand-written answers will not be accepted for reporting.

Only results and answers to the questionnaire that are reported on-line on the reporting website https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do **will be accepted.**
Directly after submitting your results and the questionnaire information on-line, you will be prompted to print the completed report form. Please sign the printed report form and return it to IRMM by fax (+32 14 571 865) or E-mail (JRC-IRMM-IMEP@ec.europa.eu). Check your results carefully before submission, since this is your final confirmation.

The deadline for submission of results is **20 May 2011**. It will not be possible to submit your results after the deadline.

Please contact JRC-IRMM-IMEP@ec.europa.eu and JRC-IRMM-MILC@ec.europa.eu **ONLY** for reporting difficulties, failures or anomalies of the online system for reporting.
For **all other issues** (communications, questions related to the content of the comparative testing round) please contact:

Diana Charels
E-mail: mbg-comparative-testing@jrc.ec.europa.eu
Phone: +39 0332 78 6518

We thank you very much for the collaboration in this comparative testing round.

Yours sincerely,

Guy Van den Eede
Head of Molecular Biology and Genomics Unit

Copy: G. Van den Eede, M. Mazzara, D. Charels, M. Maras, T. Weber, F. Ulberth (JRC).

15. Annex 3: Confirmation of shipment

Dear participant,

All test items for the third round of comparative testing have left our premises by TNT express courier this morning. For your convenience, please find hereafter the corresponding airway bill number you could refer to in order to track the relevant materials on the Web:

xxxxxxxxx

The parcel with test items that you will or have already received should contain:

- Two plastic containers each containing approximately 5 g of test item
- An acknowledgement of reception form, that should be returned to the EURL-GMFF by fax (+39 0332 789333). Should you encounter any problem with the shipment, do not hesitate to contact Eleonora Scigliano (Eleonora-Anna.SCIGLIANO@ec.europa.eu; phone +39 0332 78 58 56 +39 0332 78 58 56),
- An accompanying letter entitled '**Participation in ILC-EURL-GMFF-CT-01/11, a comparative testing round to quantify the GM content of RoundUp Ready™ soybean (soybean line 40-3-2) test items.**'.

The accompanying letter contains your **personal password** for on-line submission of your results to the reporting website

https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do

Please find herewith a pdf file of the questionnaire. This pdf file is intended as an aid in the laboratory. In the questionnaire, items with the indication (number) behind the answer box indicate that a numerical value should be given. Items bearing a question mark icon on the right-hand side contain valuable and important information for the participant. In the reporting website clicking on the icon will give access to this information. Pdf files of questionnaires bearing hand-written answers **will not be accepted**. Only results and answers to the questionnaire reported on-line to the reporting website https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do will be accepted.

The deadline for submission of your results is **20 May 2011**.

Please contact JRC-IRMM-IMEP@ec.europa.eu and JRC-IRMM-MILC@ec.europa.eu **ONLY** for reporting difficulties, failures or anomalies of the online system for reporting (i.e. https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do).

For **all other issues** (communications, questions related to the content of the comparative testing round) please contact:

Diana Charels
E-mail: mbg-comparative-testing@jrc.ec.europa.eu
Phone: +39 0332 78 6518

Please send an E-mail to me in case you have not received the above-mentioned documents. Thank you.

Kind Regards,

Eleonora

Eleonora Anna SCIGLIANO - Secretariat
European Commission - Joint Research Centre
Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit
Via E. Fermi, 2749
I - 21027 Ispra (VA)

Phone: + 39 0332 785856 + 39 0332 785856 Fax: + 39 0332 785483
E-mail: Eleonora-Anna.SCIGLIANO@ec.europa.eu
http://www.ihcp.jrc.ec.europa.eu

16. Annex 4: Acknowledgement of receipt

FAX - Record for Quality System

JRC.I.4 -MV

Date: **R71GP6/EURL** 19/07/2011 **Acknowledgement of reception** Page 1/1

Revision. 4

From :

Lab Code:

To : Molecular Biology and Genomics Unit **fax: +39 0 332 78 6159**
Method Validation / EURL-GMFF
European Commission - Joint Research Centre - IHCP
21027 ISPRA (VA) Italy File nb EURL-CT-01/11

We have received the following samples **In good condition and in dry ice** **Yes** **No**

Two plastic containers with 5 g of test item

Comments:

Date:..... Visa:.....

Please, send this document via FAX to: +39 0332 78 9333 the day of reception

This document is not a recognition of the quantity and/or quality of samples and reagents provided. This document will be used by EURL-GMFF only to confirm the reception of goods provided to participating laboratories in its Quality System.

EURL-GMFF thanks you very much for your participation.

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The Publications Office has a worldwide network of sales agents.
You can obtain their contact details by sending a fax to (352) 29 29-42758.

European Commission
EUR 25775 EN – Joint Research Centre – Institute for Institute for Health and Consumer Protection

Title: Comparative Testing Report on the Detection and Quantification of Soybean Event 40-3-2

Author(s): Diana Charels, Marko Maras, Karolina Kolodziej, Inge Verbist, Fernando Cordeiro Raposo and Marco Mazzara

Luxembourg: Publications Office of the European Union

2013 – 71 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online)

ISBN 978-92-79-28307-9 (PDF)

doi: 10.2788/82570

Abstract

In the frame of Regulation (EC) No 882/2004, the European Union Reference Laboratory for Genetically Modified Food and Feed has the duty to organise comparative testing rounds and to ensure an appropriate follow-up of these activities. This report describes the outcome of the third comparative testing round ILC-EURL-GMFF-CT-01/11. Participants had to determine the GM content in two test items denoted soybean powder levels 1 and 2, containing different GM percentages of soybean event 40-3-2 flour.

This comparative testing round was organised in collaboration with the Food Safety and Quality Unit of the Institute for Reference Materials and Measurements (Geel, BE). The soybean event 40-3-2 test items were produced in-house. The Food Safety and Quality Unit managed the on-line registration and submission of results.

A total of 155 laboratories were invited to participate in ILC-EURL-GMFF-CT-01/11. Eight National Reference Laboratories declined participation, of which one was no longer a National Reference Laboratory. One hundred and two laboratories from 43 countries returned results, of which 62 were National Reference Laboratories, 11 were members of the European Network of GMO Laboratories only, eight were only Official control laboratories and 21 were laboratories from third countries. Seven laboratories including one National Reference Laboratory, one European Network of GMO Laboratory and five laboratories from a third country did not submit results.

Participants could report the results of the exercise either in mass/mass % or in copy/copy %. In this third comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for both soybean powder levels 1 and 2 regardless of the calibration method and the measurement unit.

LB-NA- 25775-EN-N

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security, including nuclear; all supported through a cross-cutting and multi-disciplinary approach.

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