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Report on the Verification of the Performance of MON89034, 1507, MON88017, 59122 and DAS-40278-9 event-specific PCR-based Methods Applied to DNA Extracted from GM Stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize

European Union Reference Laboratory for
Genetically Modified Food and Feed

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Contact information
Name: EURL GMFF
Email: JRC-EURL-GMFF@ec.europa.eu

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EUROPEAN COMMISSION
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Directorate F – Health, Consumers and Reference Materials

Food & Feed Compliance (F.5)

Report on the Verification of the Performance of MON89034, 1507, MON88017, 59122 and DAS-40278-9 event-specific PCR-based Methods Applied to DNA Extracted from GM Stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize

25/09/2018

European Union Reference Laboratory for GM Food and Feed

Executive Summary

An application was submitted by Dow Agrosciences LLC, as represented by Dow Agrosciences Europe, to request the authorisation of genetically modified stack (GM stack) MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize (above-ground insect control-lepidopteran and coleopteran protection and weed control through herbicide tolerance to glufosinate-ammonium, glyphosate, 2,4-D and to certain aryloxyphenoxypropionate herbicides, supporting broad spectrum weed and grass control within a single field) and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003 GM Food and GM Feed. The unique identifier assigned to GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize is MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-88Ø17-3 × DAS-59122-7 × DAS-4Ø278-9.

The GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize has been obtained by conventional crossing between the genetically modified maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9, without any new genetic modification.

The EURL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events MON89034, 1507, MON88017, 59122 and DAS-40278-9 (see http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx). In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EURL GMFF has carried out only an *in-house* verification of the performance of each validated method when applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

The results of the in-house verification led to the conclusion that the individual methods meet the ENGL performance criteria also when applied to genomic DNA extracted from the GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

This report is published at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx.

Content

Quality assurance

The EURL GMFF is ISO 17025:2005 accredited [certificate number: Belac 268 TEST (Flexible Scope for DNA extraction, DNA identification and real Time PCR) and ISO 17043:2010 accredited (certificate number: Belac 268 PT, proficiency test provider).

The original version of the document containing evidence of internal checks and authorisation for publication is archived within the EURL GMFF quality system.

Address of contact laboratory:

European Commission
Directorate General Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for GM Food and Feed
Food & Feed Compliance (F.5)
Via E. Fermi, 2749. TP201
I-21027 Ispra (VA), Italy

Functional mailbox: JRC-EURL-GMFF@ec.europa.eu

1. Introduction

The EU legislative system [1, 2] for genetically modified food and feed foresees that any GMO for food and feed use shall undergo the authorisation process before it can be placed on the market. This holds true also for a GMO containing more than one single GM event obtained by conventional crossing, co-transformation or re-transformation (genetically modified stack).

Consequently, the application for authorisation of a GM stack shall be accompanied, among others, by an event-specific method for detection, identification and quantification for each GM event composing the stack, and by samples of the stack and food and feed derived from it. The EURL GMFF shall validate the event-specific methods of detection proposed by the applicant with regard to their performance when applied to DNA extracted from the stack, and shall report to the European Food Safety Authority, who will include the EURL GMFF report in the overall opinion concerning the risk assessment and potential authorisation of the assessed stack. In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EURL GMFF carries out an *in-house* verification of the performance of each event-specific method if this method has previously been validated by the EURL GMFF for the parental single-line event and these events have been stacked by conventional crossing. These criteria are met for the GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Upon reception of methods, samples and related data (step 1), the EURL GMFF carried out the assessment of the documentation (step 2) and the *in-house* verification of the methods (step 3) according to the requirements of Regulation (EU) No 503/2013 (Annex III).

The results of the *in-house* verification study were evaluated with reference to ENGL method performance requirements and to the validation results on the individual events.

2. Step 1 (dossier reception and acceptance)

Dow Agrosciences LLC submitted the detection methods, data demonstrating their adequate performance when applied to genomic DNA extracted from the stack, and the corresponding control samples of DNA extracted from the GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize and from non-GM maize.

The dossier was found to be complete and thus was moved to step 2.

3. Step 2 (dossier scientific assessment)

The data provided by the applicant were assessed against the method acceptance criteria set out by the ENGL [3] and with regard to their documentation and reliability.

Table 1 shows values of trueness (expressed as bias %) and precision (expressed as RSD_r %) calculated by the applicant for the five methods applied to MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize. Means are the average of fifteen replicates obtained through 3 runs performed with ABI Prism® 7900HT real-time PCR equipment. Percentages are expressed as GM DNA / total DNA x 100.

Note: Numerical values presented in the following tables were rounded keeping two digits for values ≤ 1, one digit for values between 1 and 10 and no digit for values ≥ 10. The calculations in the MS Excel files however were done over not rounded data. This approach might create small inconsistencies in the numerical values reported in the tables but it allows a higher precision in the final results.

Table 1. Trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSD_r %) provided by the applicant for the MON89034, 1507, MON88017, 59122 and DAS-40278-9 methods applied to GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

MON89034*				
Sample GM%	**Expected value (GMO %)**			
	0.085	**0.9**	**5.0**	**10**
Mean	0.086	0.909	5.36	9.48
RSD_r (%)	15.3	10.1	8.2	11.6
Bias (%)	1.2	1.0	7.2	-5.2
1507				
Sample GM%	**Expected value (GMO %)**			
	0.085	**0.9**	**2.0**	**5.0**
Mean	0.081	0.863	1.99	4.82
RSD_r (%)	16.9	7.1	7.0	3.5
Bias (%)	-4.7	-4.1	-0.5	-3.6
MON88017				
Sample GM%	**Expected value (GMO %)**			
	0.085	**0.9**	**5.0**	**10**
Mean	0.095	0.977	5.79	10.13
RSD_r (%)	12.4	8.9	14.9	5.9
Bias (%)	11.8	8.6	14	1.3
59122				
Sample GM%	**Expected value (GMO %)**			
	0.085	**0.9**	**2.0**	**5.0**
Mean	0.064	0.753	1.7	5.14
RSD_r (%)	21.3	15.4	10.6	11.5
Bias (%)	-24.7	-16.3	-15	2.8

DAS-40278-9				
Sample GM%	**Expected value (GMO %)**			
	0.08	**0.9**	**2.0**	**5.0**
Mean	0.068	0.824	1.93	4.93
RSD_r (%)	7.5	5.1	3.6	3.9
Bias (%)	-15.0	-8.4	-3.5	-1.4

* Numbers are not rounded but are presented as reported by the applicant

The EURL GMFF verified the data and concluded that they seemed to confirm that the methods meet the ENGL performance criteria [(3)].

Four requests of complementary information regarding control samples (purity, quality and parental origin), DNA sequences and robustness were addressed to the applicant.

Concerning the robustness of MON89034 and MON88017 methods the applicant provided the conclusion that these methods are 'really susceptible to variations of the annealing temperature'. For the other clarifications requested the EURL GMFF verified the data and the complementary information received and accepted the received clarifications as satisfactory.

The dossier was therefore moved to step 3.

4. Step 3 (EURL GMFF experimental testing)

In step 3 the EURL GMFF implemented the five methods in its own laboratory and performed a verification of their performance when applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

4.1 Materials

The following control samples were provided by the applicant:

- genomic DNA extracted from homogenised hybrid hemizygous seeds of GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, as positive control sample.
- genomic DNA extracted from homogenised seeds of conventional (non-GM) maize whose genetic background (SLB01 and BE9514XT) is comparable to that of the positive control sample, as negative control sample.

The EURL GMFF prepared test samples of different GMO concentrations by mixing genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize with non-GM maize genomic DNA in a constant amount of total maize genomic DNA. The same GM concentrations as in the validation of the methods for the single lines were achieved. Table

2 shows the five GM concentrations used in the verification of the MON89034, 1507, MON88017, 59122 and DAS-40278-9 methods when applying them to genomic DNA extracted from the GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Table 2. Percentage (GM%) of MON89034, 1507, MON88017, 59122 and DAS-40278-9 in MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 stack genomic DNA contained in the verification samples.

MON89034 GM%* (GM DNA/ total maize DNA x 100)	**1507 GM%*** (GM DNA/ total maize DNA x 100)	**MON88017 GM%*** (GM DNA/ total maize DNA x 100)	**59122 GM%*** (GM DNA/ total maize DNA x 100)	**DAS-40278-9 GM%*** (GM DNA/ total maize DNA x 100)
0.09	0.10	0.09	0.10	0.10
0.40	0.50	0.50	0.40	0.40
0.90	0.90	0.90	0.90	0.90
3.0	2.0	5.0	2.0	2.0
8.0	5.0	8.0	4.5	5.0

*(percentage expressed in copy number ratio)

The protocols described by the applicant were implemented precisely in the EURL GMFF laboratory and were in accordance with the protocols already published for the individual 1507, and DAS-40278-9 GM events (available at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx). Some deviations were introduced for 59122, MON89034 and MON88017 methods as described in 4.4.1.

4.2 DNA extraction

A method for DNA extraction from maize was previously evaluated by the EURL GMFF with regard to its performance characteristics and was considered valid, i.e. fit the purpose of providing maize DNA of appropriate quality and amount for being used in subsequent PCR experiments.

On a general note the EURL GMFF recommends that laboratories using this validated method for testing complex or difficult matrices always verify that the extracted genomic DNA is of sufficient quality.

The protocol for the DNA extraction method is available at http://gmo-crl.jrc.ec.europa.eu/summaries/TC1507-DNAextrc.pdf.

Consequently, the EURL GMFF did not verify the DNA extraction method proposed by the applicant.

4.3 Experimental design

Eight PCR runs for each method were carried out. In each run, samples were analysed in parallel with both the GM-specific system and the reference system High Mobility Group (*hmg*) gene. Five GM levels were examined per run, for each GM level in duplicate. PCR analysis was performed in triplicate for all samples. In total, for each method, MON89034, 1507, MON88017, 59122 and DAS-40278-9, the quantification of the five GM levels was performed

as an average of sixteen replicates per GM level (8 runs x 2 replicated levels per run). An Excel spreadsheet was used for the determination of the GM%.

4.4 PCR methods

During the verification study, the EURL GMFF carried out parallel tests on DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize using the single detection methods previously validated for the respective single GM events MON89034, 1507, MON88017, 59122 and DAS-40278-9.

For detection of GM maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9, DNA fragments of 77-bp, 58-bp, 95-bp, 86-bp and 98-bp respectively are amplified using specific primers. PCR products are measured during each cycle (real-time) by means of target-specific oligonucleotide probes labelled with two fluorescent dyes: FAM (6-carboxyfluorescein) as reporter dye at their 5′-end and TAMRA (6-carboxytetramethylrhodamine) as a quencher dye at their 3′-end for 1507, MON88017, DAS-59122-7, DAS-40278-9 and FAM as a reporter dye at its 5′ end and MGBNFQ (minor groove binding non-fluorescent quencher) as a quencher dye at its 3′ end for MON89034.

For quantification of GM maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9, a taxon-specific reference system amplifies a 79-bp fragment of *hmg* gene, a maize endogenous gene (GenBank AJ131373), using two *hmg* gene-specific primers and a gene-specific probe labelled with FAM and TAMRA.

For the relative quantification of GM maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9 standard curves are generated both for the MON89034, 1507, MON88017, 59122 and DAS-40278-9 systems and for the *hmg* specific system by plotting Cq values of the calibration standards against the logarithm of the DNA amount and by fitting a linear regression into these data. Thereafter, the Cq values of the unknown samples are measured and, by means of the regression formula, the relative amount of MON89034, 1507, MON88017, 59122 and DAS-40278-9 DNA is estimated.

For detailed information on the preparation of the respective standard curve calibration samples please refer to the protocols of the validated methods at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx.

4.4.1 Deviations from the validated methods

During the verification study on DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, the EURL GMFF applied a deviation from the validated 59122 event-specific method. This deviation consists in the adjusting the pH of the commercial buffer II (Applied Biosystems 4379878) to 8.0 instead of pH 8.3 (as originally

validated) and it was applied previously in the context of the verification of the method on DNA extracted from maize GM stack Bt11 x 59122-7 x MIR604 x TC1507 x GA21.

For MON89034 and MON88017 methods, the buffer to be used with the *hmg* reference system is discontinued (TaqMan® buffer A, Applied Biosystems). Therefore, the EURL GMFF decided to maintain all conditions in accordance with the initial validation, i.e. primers and probes, volume and cycling conditions, but to use TaqMan® Universal Master Mix. In fact, the EURL GMFF implemented the *hmg* reference system as validated in the context of the dossier CRLVL04/09 (page 8/10 of http://gmo-crl.jrc.ec.europa.eu/summaries/2012-01-27_MON87460_validated_Method.pdf).

For the other methods no deviations from the original validated methods were introduced.

4.5 Results

Tables 3 to 7 present the values of the slope of the different standard curves generated by the EURL GMFF when using DNA extracted from the GM stack, from which the PCR efficiency is calculated using the formula $[10^{(-1/slope)} - 1] \times 100$, and of the coefficient of determination (R^2) reported for all PCR systems in the eight runs, for GM maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9. Slope values and R^2 coefficient values were rounded to two digits.

Table 3. Values of standard curve slope, PCR efficiency and R^2 coefficient for the MON89034 method on GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Run	MON89034			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.55	91	1.00	-3.41	97	1.00
2	-3.51	93	1.00	-3.39	97	1.00
3	-3.58	90	1.00	-3.39	97	1.00
4	-3.49	93	1.00	-3.41	97	1.00
5	-3.57	91	1.00	-3.43	96	1.00
6	-3.48	94	1.00	-3.45	95	1.00
7	-3.54	92	1.00	-3.37	98	1.00
8	-3.53	92	1.00	-3.36	98	1.00
Mean	-3.53	92	1.00	-3.40	97	1.00

Table 4. Values of standard curve slope, PCR efficiency and R^2 coefficient for the 1507 method on GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Run	1507			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.09	111	0.99	-3.08	111	1.00
2	-3.22	104	1.00	-3.20	105	1.00
3	-3.17	107	0.99	-3.17	107	1.00
4	-3.10	110	1.00	-3.24	103	1.00
5	-3.19	106	0.99	-3.11	110	1.00
6	-3.29	101	0.99	-3.27	102	1.00
7	-3.09	111	0.96	-3.15	108	1.00
8	-3.21	105	0.99	-3.23	104	1.00
Mean	-3.17	107	0.99	-3.18	106	1.00

Table 5. Values of standard curve slope, PCR efficiency and R^2 coefficient for the MON88017 method on GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Run	MON88017			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.29	101	1.00	-3.38	98	1.00
2	-3.25	103	1.00	-3.37	98	1.00
3	-3.39	97	1.00	-3.39	97	1.00
4	-3.38	98	1.00	-3.40	97	1.00
5	-3.45	95	1.00	-3.36	98	1.00
6	-3.30	101	1.00	-3.37	98	1.00
7	-3.34	99	1.00	-3.39	97	1.00
8	-3.36	99	1.00	-3.40	97	1.00
Mean	-3.34	99	1.00	-3.38	98	1.00

Table 6. Values of standard curve slope, PCR efficiency and R^2 coefficient for the 59122 method on GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Run	59122			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.35	99	1.00	-3.28	102	1.00
2	-3.49	93	1.00	-3.24	104	1.00
3	-3.43	96	1.00	-3.21	105	1.00
4	-3.23	104	0.99	-3.30	101	1.00
5	-3.43	96	1.00	-3.24	104	1.00
6	-3.39	97	0.99	-3.34	99	1.00
7	-3.34	99	1.00	-3.20	105	1.00
8	-3.45	95	1.00	-3.31	101	1.00
Mean	-3.39	97	1.00	-3.26	103	1.00

Table 7. Values of standard curve slope, PCR efficiency and R^2 coefficient for the DAS-40278-9 method on GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Run	DAS-40278-9			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.35	99	1.00	-3.38	98	1.00
2	-3.41	96	1.00	-3.42	96	1.00
3	-3.36	99	1.00	-3.42	96	1.00
4	-3.35	99	1.00	-3.38	98	1.00
5	-3.37	98	1.00	-3.39	97	1.00
6	-3.42	96	1.00	-3.37	98	1.00
7	-3.44	95	1.00	-3.42	96	1.00
8	-3.37	98	1.00	-3.38	98	1.00
Mean	-3.38	98	1.00	-3.39	97	1.00

The mean PCR efficiencies of the GM and species-specific systems were above 90% (92% and 97% for MON89034 and *hmg* systems, respectively, 107% and 106% for 1507 and *hmg*, 99% and 98% for MON88017 and *hmg*, 97% and 103% for 59122 and *hmg*, 98% and 97% for DAS-40278-9 and *hmg*). The mean coefficient of determination (R^2) of the methods was 1.00 for all systems in all cases, except for 1507 GM system (R^2 = 0.99). The data presented in Tables 3 to 7 confirm the appropriate performance characteristics of the five methods when tested on genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize in terms of PCR efficiency and coefficient of determination.

The EURL GMFF also assessed the values of trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSD_r %) of the five methods applied to

samples of DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, see tables 8 to 12.

Table 8. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MON89034 method applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

MON89034					
Unknown sample GM%	**Expected value (GMO%)**				
	0.09	**0.40**	**0.9**	**3.0**	**8.0**
Mean	0.10	0.43	0.98	3.2	8.1
SD	0.01	0.03	0.04	0.14	0.48
RSD_r (%)	10	6.9	4.6	4.4	5.9
Bias (%)	14	8.3	9.4	6.8	1.0

Table 9. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the 1507 method applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

1507					
Unknown sample GM%	**Expected value (GMO%)**				
	0.10	**0.50**	**0.90**	**2.0**	**5.0**
Mean	0.08	0.42	0.78	1.7	4.8
SD	0.01	0.05	0.06	0.16	0.31
RSD_r (%)	15	11	8.1	9.2	6.5
Bias (%)	-19	-17	-14	-13	-4,0

Table 10. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MON88017 method applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

MON88017					
Unknown sample GM%	**Expected value (GMO%)**				
	0.09	**0.50**	**0.90**	**5.0**	**8.0**
Mean	0.09	0.50	0.92	5.3	8.0
SD	0.01	0.04	0.07	0.21	0.25
RSD_r (%)	11	7.7	8.0	4.0	3.2
Bias (%)	0.33	0.94	1.7	6.0	-0.42

Table 11. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the 59122 method applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

59122					
Unknown sample GM%	**Expected value (GMO%)**				
	0.10	**0.40**	**0.9**	**2.0**	**4.5**
Mean	0.09	0.34	0.83	1.8	4.2
SD	0.01	0.05	0.06	0.17	0.36
RSD_r (%)	11	16	6.8	9.8	8.7
Bias (%)	-8.4	-15	-7.9	-11	-7.2

Table 12. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the DAS-40278-9 method applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

DAS-40278-9					
Unknown sample GM%	**Expected value (GMO%)**				
	0.10	**0.40**	**0.90**	**2.0**	**5.0**
Mean	0.09	0.42	0.92	2.1	5.1
SD	0.01	0.03	0.04	0.13	0.32
RSD_r (%)	7.8	7.0	4.1	6.3	6.3
Bias (%)	-0.11	4.8	2.5	4.1	2.7

The trueness of the method is estimated using the measurements of the method bias for each GM level. According to the ENGL acceptance criteria and method performance requirements, the trueness of the method should be less or equal to ± 25% across the entire dynamic range. As shown in Tables 8 to 12, the values range from 1.02% to 14% for MON89034, from -19% to -4,0% for 1507, from -0.42% to 6.0% for MON88017, -15% to -7,2% for 59122 and from -0.11% to 4.8% for DAS-40278-9. Therefore, the five methods satisfy the above mentioned requirement throughout their respective dynamic ranges, also when applied to DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

Tables 8 to 12 also show the relative repeatability standard deviation (RSD_r) as estimated for each GM level. According to the ENGL acceptance criteria and method performance requirements, the EURL GMFF requires RSD_r values to be equal or below 25%. As the values range between 4.4% and 10% for MON89034, between 6.5% and 15% for 1507, between 3.2% and 11% for MON88017, between 6.8% and 16% for 59122 and between 4.1% and 7.8% for DAS-40278-9, the five methods satisfy this requirement throughout their respective dynamic ranges when applied to DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize.

5. Conclusions

The performance of the five event-specific methods for the detection and quantification of maize single line events MON89034, 1507, MON88017, 59122 and DAS-40278-9 when applied to genomic DNA extracted from GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize, meets the ENGL performance requirements, as assessed on the control samples provided by the applicant.

Therefore these methods, developed and validated to detect and quantify the single maize events MON89034, 1507, MON88017, 59122 and DAS-40278-9 can be equally applied for the detection and quantification of the respective events in genomic DNA extracted from the GM stack MON89034 × 1507 × MON88017 × 59122 × DAS-40278-9 maize or any of its sub-combinations, provided that sufficient genomic DNA of appropriate quality is available.

6. References

1. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance). OJ L 268, 18.10.2003, p. 1–23.

2. Regulation (EU) No 503/2013 of 3 April 2013 "on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006".

3. European Network of GMO Laboratories (ENGL), 'Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing' 2008, http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf

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