Collaborative Validation of an Event-Specific Quantitative Real-Time PCR Method for Genetically Modified Rice Event TT51-1 Detection

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Ⓢ Supporting Information

ABSTRACT: In this study, a collaborative trial of validating a real-time PCR method for the TT51-1 rice event was organized, including six participating laboratories. In this validation, serially diluted solutions from homogeneous genomic DNA of the TT51-1 event were used to construct standard curves of the TT51-1 event and phospholipase D (*PLD*) assays. The PCR efficiency was 95%, and the R^2 coefficient was 0.99 for the TT51-1 system. The mean quantitative values for blind samples containing 0.1%, 0.5% 1%, 5%, and 10% (w/w) TT51-1 corresponded to 0.1%, 0.51%, 1.06%, 4.83%, and 9.62%, respectively, with a bias (%) ranging from −3.77 to 5.87%. The repeatability and reproducibility were all below 25% across the entire dynamic range. Furthermore, the measurement uncertainties of the quantitative results were estimated to be 0.10%, 0.20%, 0.40%, 1.76%, and 3.52% (w/w) for the tested samples. Both the LOD and LOQ were calculated to be 0.22%. This collaborative trial demonstrated that the TT51-1 method produces reliable, comparable, and reproducible results for a given sample set and can be adopted as a detection standard for testing laboratories.

KEYWORDS: *genetically modified rice, TT51-1, collaborative trial, quantitative real-time PCR*

■ INTRODUCTION

As of 2012, genetically modified (GM) crops have been planted in 28 countries and approved for import for food and feed use and for release into the environment in 60 countries worldwide.[1] A total of 319 events for 25 crops have been approved for commercialization, of which maize has the most events approved (121), followed by cotton (48), potato (31), canola (30), and soybean (22).[1] Although the commercialization of genetically modified crops is accelerating, the public still has doubts about the safety of GM products. Since consumers are very concerned about the presence of transgenic components in food products, many countries or regions have instituted regulations that stipulate food or feed containing genetically modified organisms (GMO) must be labeled when the threshold value is reached, and food or feed containing unauthorized GMOs is not permitted to be sold in markets.[2−5] For instance, labeling thresholds of 0.9%, 3%, and 5% are stipulated in the European Union (EU), Korea, and Japan, respectively.[2−5] At present, a zero-tolerance policy is implemented based on qualitative PCR technology in China, prohibiting unapproved GM varieties from sale.[6]

The implementation of a labeling policy requires the establishment and application of reliable detection and quantitative analytical methods. For analysis of samples, the adopted GMO detection method requires validated procedures to ensure that it can produce reliable and repeatable results before adoption as an analytical tool for GMO determination. Therefore, it is prescribed by legislation that the adopted detection method

should be fully validated by collaborative trial in the EU, China, and some other countries.[7,8] At present, the internationally accepted guidelines mostly refer to ISO 5725 and the IUPAC (International Union of Pure and Applied Chemistry) protocol for full validation of an analytical method by collaborative study.[9,10] The European Network of GMO laboratories (ENGL) issued a document defining the minimum performance requirements for PCR-based analytical methods of GMO testing according to the basic principles and requirements of ISO5725 and/or IUPAC.[11]

To provide reliable and harmonized analytical methods for GMO detection, the European Commission published a "GMOMETHODS" database supplying validated methods for GMO detection.[12] The GMOMETHODS database has collected 118 different PCR methods allowing identification of 51 single GM events, 18 taxon-specific genes, and eight screening elements commonly used for the development of GMOs.[12,13] Most of the reported collaborative trials of GMO detection methods were organized and performed by the European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF).[12] In addition, ring trials of the tomato (*LAT52*) and rice (*SPS*) reference genes were organized independently by Shanghai Jiao Tong University, China.[14,15]

Table 1. Primers and Probes Used

PCR assay	name	sequence	reference
PLD real-time PCR	KVM159	TGGTGAGCGTTTTGCAGTCT	Mazzara et al., 2006[27]
	KVM160	CTGATCCACTAGCAGGAGGTCC	
	TM013	FAM-TGTTGTGCTGCCAATGTGGCCTG-BHQ1	
TT51-1 real-time PCR	TT511C	GCGTCCAGAAGGAAAAGGAATA	Wu et al., 2010[30]
	TT511G	AGAGACTGGTGATTTCAGCGGG	
	TT511P	FAM-ATCTGCCCCAGCACTCGTCCG-BHQ1	

Rice (*Oryza sativa*) is one of the most important food crops worldwide, and rice production is spread over many countries and regions outside the Antarctic; nearly half of the world's population live on rice as their staple food.[16] A large amount of transgenic research has been carried out on rice throughout the world,[17] but up until 2012, only seven transgenic rice cultivars were approved for cultivation or food/feed use according to the information of GM Approval Database published by International Service for the Acquisition of Agri-Biotech Applications (ISAAA).[18] The commercial transgenic rice cultivars involve an antiallergy event 7Crp#10 cultivated in Japan;[19] three insect-resistance events, GM Shanyou 63 and Huahui-1/TT51-1 planted in China[20,21] and Tarom molaii + cry1Ab in Iran;[22] and three herbicide-tolerance events, LLRICE06, LLRICE601, and LLRICE62 developed by Bayer CropScience.[23−25] GM Shanyou 63 is a hybrid cultivated with Huahui-1/TT51-1 as parental line, which was granted the safety certificate by China in 2009.[26] Because of the important position of rice in the daily diet, people pay more attention to the detection of genetically modified ingredients in rice and rice products. For the approved rice events, only the LLRice62 event has been reported by a validated event-specific quantitative detection method.[27]

TT51-1 is an insect-resistant transgenic rice event harboring a hybrid Cry1Ab/Ac gene driven by the rice *actin 1* gene promoter and the nopaline synthase (NOS) terminator.[28,29] To monitor the planting and production of TT51-1-derived rice varieties, an event-specific detection method has also been established for quantification of the TT51-1 event.[30] Method validation is an essential step of standardization for GMO testing methods so that a GMO testing laboratory can produce reliable analytical results. In this study, we report a collaborative ring trial for validating the TT51-1 event-specific quantitative detection method.

■ MATERIALS AND METHODS

Materials. Genuine seeds of a homozygous line of GM rice (*O. sativa*) containing the TT51-1 event and the recipient material for TT51-1, nontransgenic *O. sativa* cv. Minghui 63, were germinated in a greenhouse, and the young leaves were collected for large-scale genomic DNA extraction.

To exclude the confounders contributing to method variability, such as sample grinding and DNA extraction, the pure genomic DNA was directly used to prepare blind samples. Five blind samples representing five TT51-1 content levels, corresponding to 0.1%, 0.5%, 1%, 5%, and 10% (weight/weight), were prepared by mixing TT51-1 genomic DNA with non-GM rice genomic DNA. The concentration of TT51-1 and non-GM rice DNA was first adjusted to 20 ng/μL with a 0.1× TE solution. Then 100 μL of TT51-1 genomic DNA and 900 μL of non-GM rice DNA were put together into a 2 mL centrifuge tube and mixed well to obtain sample of 10% level. Then, a 10% TT51-1 solution was further used to perform a serial dilution with non-GM rice DNA to successively obtain 5%, 1%, 0.5% and 0.1% TT51-1 solutions. After finishing the dilution, the prepared sample solutions were dispensed into 1.5 mL tubes for further application with 100 μL per tube.

DNA Extraction. DNA samples from TT51-1 leaves were extracted using the CTAB method according to ISO 21571.[31] The details of the DNA extraction protocol are the same as in a previously published paper.[14] DNA purity and concentration were estimated by agarose gel electrophoresis and the ultraviolet spectrometric method with a Nano-Drop 1000 UV/vis spectrophotometer (NanoDrop, Wilmington, DE, USA) according to ISO 21571. Meanwhile, DNA concentrations were further measured using the Picogreen dye method in a VersaFluor fluorometer system (Bio-Rad, Hercules, CA, USA). The DNA samples were used for the ring trial if the measured concentrations were similar using the two methods. The concentrations of all DNA samples were adjusted to 20 ng/μL for further experiment analysis.

Real-Time PCR. An event-specific detection method for TT51-1 was established by Wu et al. using primers together with a probe annealing to the 3′ junction fragment between the insert DNA and genomic DNA of TT51-1, producing an amplicon of 120 bp.[30] As in their published paper, the *PLD* gene, which has been collaboratively validated by 11 laboratories from eight countries of the EU, was selected as the rice reference gene.[27] PCR primers and probes are shown in Table 1. The probe contains a reporter dye (FAM) at the 5′ end and a quencher dye (BHQ1) at the 3′ end.

The real-time PCR assay for the *PLD* gene was performed in a volume of 25 μL containing 20 ng of genomic DNA as template, 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 200 nM each of primers KVM159 and KVM160, and 200 nM probe TM013. The real-time PCR assay for TT51-1 was also performed in a volume of 25 μL containing 20 ng of genomic DNA as template, 1× TaqMan Universal PCR Master Mix, 800 nM each of primers TT511 V and TT511G, and 400 nM probe TT511P.

All real-time PCR reactions were carried out as follows: a pre-digestion step of 50 °C for 2 min, an initial denaturation and UNG deactivation step of 95 °C for 10 min, and then 50 cycles of 94 °C for 15 s (denaturation) and 60 °C for 1 min (annealing and extension). Fluorescence measurements were performed during the annealing and extension step. The above PCR reaction was pretested to ensure it was fit for various fluorescence thermal cyclers, such as the Opticon 2 (MJ Research, Waltham, MA, USA), the Prism ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), and the Rotor Gene 3000A (Corbett Robotics, Australia).

Collaborative Trials. The ring trial included six GMO detection laboratories (three laboratories from the Ministry of Agriculture, China, and the others from the General Administration of Quality Supervision, Inspection and Quarantine of China). Each laboratory received seven genomic samples: one sample labeled as TT51-1 was used to construct standard curves after being serially diluted, one sample labeled as Minghui 63 was used as a negative control, and five samples labeled S1, S2, S3, S4, and S5 were used as blind samples, representing TT51-1 content levels of 0.1%, 0.5%, 1%, 5%, and 10%, respectively. Each sample contained a 100 μL volume at a concentration of 20 ng/μL. The genomic DNA samples and primers/probe were stored in a single closed box filled with dry ice and mailed to each participating laboratory.

The DNA sample TT51-1 was serially diluted to concentrations of 10, 2.35, 0.235, 0.0235, and 0.00235 ng/μL by adding ddH$_2$O, marked R1−R5. The serial solutions R1−R5 were used for calibration to set standard curves. In the quantitative PCR assays, 2 μL of R1−R5 (20 ng total DNA) was added to the real-time PCR reaction systems, each corresponding to 42 500, 10 000, 1000, 100, or 10 copies. The blind samples (S1−S5) were diluted 2-fold by adding ddH$_2$O, and
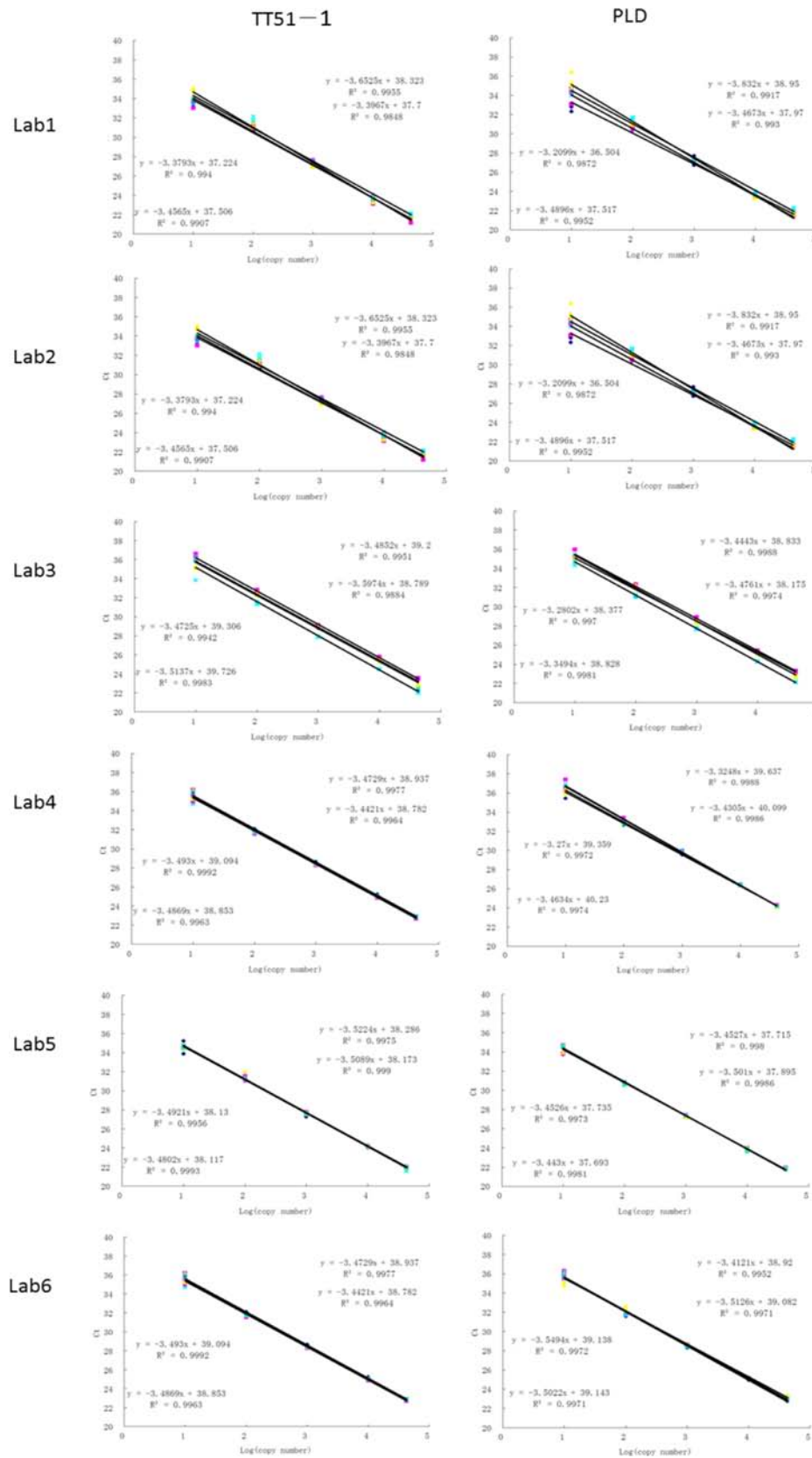
Figure 1. Separate standard curves for the TT51-1 target sequence and the rice *PLD* reference sequence were established by plotting Ct values against the log values of initial template copies in the PCR reactions, using serially diluted reference solutions from 100% TT51-1 material. Lab1–Lab6 correspond to TT51-1 and *PLD* gene standard curves constructed according to the calibration Ct values provided by six participating laboratories; each laboratory carried out four replications for the TT51-1 and *PLD* assays.

Table 2. Slope, PCR Efficiency, and R^2 Values of the Standard Curves

lab	rep	TT51-1			PLD		
		slope	PCR efficiency	linearity (R^2)	slope	PCR efficiency	linearity (R^2)
Lab 1	1	−3.05	113%	1.00	−3.26	103%	1.00
	2	−3.23	104%	0.99	−3.38	98%	0.99
	3	−3.22	105%	0.98	−3.37	98%	1.00
	4	−3.40	97%	0.99	−3.45	95%	0.99
Lab 2	1	−3.38	98%	0.99	−3.21	105%	0.99
	2	−3.46	95%	0.99	−3.49	93%	0.99
	3	−3.65	88%	1.00	−3.83	82%	0.99
	4	−3.40	97%	0.98	−3.47	94%	0.99
Lab 3	1	−3.47	94%	0.99	−3.28	102%	1.00
	2	−3.51	93%	1.00	−3.35	99%	1.00
	3	−3.49	94%	0.99	−3.44	95%	1.00
	4	−3.60	90%	0.99	−3.48	94%	1.00
Lab 4	1	−3.49	93%	1.00	−3.27	102%	1.00
	2	−3.49	94%	1.00	−3.46	94%	1.00
	3	−3.47	94%	1.00	−3.32	100%	1.00
	4	−3.44	95%	1.00	−3.43	96%	1.00
Lab 5	1	−3.49	93%	1.00	−3.45	95%	1.00
	2	−3.48	94%	1.00	−3.44	95%	1.00
	3	−3.52	92%	1.00	−3.45	95%	1.00
	4	−3.51	93%	1.00	−3.50	93%	1.00
Lab 6	1	−3.49	93%	1.00	−3.55	91%	1.00
	2	−3.49	94%	1.00	−3.50	93%	1.00
	3	−3.47	94%	1.00	−3.41	96%	0.99
	4	−3.44	95%	1.00	−3.51	93%	1.00
	mean	−3.44	95%	0.99	−3.43	96%	1.00

2 μL of S1−S5 was delivered into the real-time PCR reactions. On each PCR plate, the samples (R1−R5, S1−S5) were amplified for both the TT51-1-specific assay and the PLD-specific assay at the same time, and the negative and blank controls were included on the same plate. Each sample was run three times in parallel, and each plate required four replications per participating laboratory.

Data Analysis. The participants were asked to record the Ct values of all samples at the end of the real-time PCR amplification and send back their records containing all 240 Ct values [2 genes × (5 calibrators + 5 samples) × 3 parallels × 4 repeats = 240] within a specified time. The returned data were analyzed using Microsoft Excel software to determine the characteristics of the TT51-1-specific method. The PCR efficiency, linearity of regression, accuracy, repeatability, and reproducibility were calculated according to the requirements of ISO 5725 and the validation reports of GMO testing methods reported by EU-RL GMFF.[9,13] The measurement uncertainty, limits of detection (LOD), and limits of quantification (LOQ) of the TT51-1 system were estimated on the basis of the performance data provided by the six participants, according to the Guidance Document issued by the European Commission Joint Research Center, Institute for Reference Materials and Measurements (JRC-IRMM).[32]

■ RESULTS AND DISCUSSION

PCR Efficiency and Linearity. All participants sent back test Ct values of the serial reference solutions within a specified time with no missing data. Separate standard curves for the TT51-1 and PLD assays were generated by plotting the returned Ct values against the logarithm of the copy number in the reference solutions (R1−R5), with copy numbers ranging from 425 000 to 10 (Figure 1). Good linearity was observed between Ct values and copy numbers of templates in the calibration curves for the TT51-1 and PLD assays. The values of the slopes and the regression coefficients (R^2 value) for the TT51-1 and PLD assays were all within the allowed range according to the requirements for GMO analytical methods

issued by EU-CRL,[11] as shown in Table 2. The mean R^2 coefficient was 1.00 for the PLD detection method and 0.99 for the TT51-1 detection method; the linearities of both methods were more than the minimum acceptable value of 0.98.

The mean slope of all the standard curves from six participants was −3.43 for the PLD assay and 3.44 for the TT51-1 assay, within the range of −3.1 to −3.6. The PCR efficiency was calculated on the basis of the formula Efficiency = $(10^{(−1/\text{slope})} − 1) \times$ 100 and is shown in Table 2. The mean PCR efficiency was 96% for the PLD assay and 95% for the TT51-1 assay. The results revealed that the TT51-1 detection method had high PCR efficiency and good linearity between Ct values and copy numbers of TT51-1 templates.

Quantification of Blind Samples. In this trial, five samples (S1−S5), with GM contents of 0.1%, 0.5% 1%, 5%, and 10%, respectively, were used as blind samples to validate the TT51-1 event-specific real-time PCR systems. All the participants returned the four replicated results of the five blind samples except for Lab 3. Lab 3 reported only three replicate test results at the 0.1% GM level (sample S1), but provided four replicate test results at the other GM levels. All the returned data were used for further statistical analysis. The copy numbers of TT51-1 event genome DNA and total rice genome DNA in the blind samples were calculated according to the returned mean Ct value and the constructed standard curves of the TT51-1 and PLD assay, respectively. The GM contents of the blind samples were determined by the formula GM% = (copy number of TT51-1/ copy number of PLD) × 100. The calculated GM content of the five samples from each participating laboratory is listed in Table 3. Cochran's test and Grubbs' test were performed to check for significant outliers of estimated values according to ISO 5725-2.[9] No outlier was found through Grubbs' test, but two values at the 1.0% GM level and one value each at the 5.0% and 10.0%

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Table 3. Determined GM% Values of the Six Participants for the Five Unknown Samples

| | sample GMO content (GM% = GM copy number/genome copy number × 100) | | | | | | | | | | | | | | | | | | | |
| | 0.10% | | | | 0.50% | | | | 1.00% | | | | 5.00% | | | | 10.00% | | | |
Lab	rep 1	rep 2	rep 3	rep 4	rep 1	rep 2	rep 3	rep 4	rep 1	rep 2	rep 3	rep 4	rep 1	rep 2	rep 3	rep 4	rep 1	rep 2	rep 3	rep 4
Lab 1	0.06%	0.07%	0.06%	0.09%	0.79%	0.39%	0.66%	0.53%	2.44%[a]	0.79%	0.75%	0.86%	6.03%	3.20%	4.51%	10.38%[a]	30.33%[a]	5.39%	5.98%	10.33%
Lab 2	0.09%	0.10%	0.17%	0.11%	0.44%	0.59%	0.71%	0.49%	0.72%	0.68%	1.00%	2.88%[a]	3.22%	5.89%	6.57%	6.24%	8.59%	9.35%	13.73%	10.48%
Lab 3	0.08%	0.10%	NR	0.12%	0.45%	0.36%	0.48%	0.52%	0.93%	0.97%	1.23%	1.21%	4.94%	4.94%	4.28%	4.79%	9.03%	7.82%	10.30%	9.35%
Lab 4	0.10%	0.09%	0.13%	0.07%	0.35%	0.41%	0.52%	0.43%	0.95%	0.87%	1.00%	0.79%	4.27%	3.94%	4.21%	3.80%	8.91%	7.53%	8.15%	6.91%
Lab 5	0.11%	0.12%	0.11%	0.11%	0.46%	0.54%	0.61%	0.48%	1.33%	0.99%	1.23%	0.93%	2.04%	4.19%	6.70%	5.75%	12.20%	10.37%	10.56%	8.92%
Lab 6	0.12%	0.11%	0.09%	0.10%	0.65%	0.51%	0.50%	0.47%	1.44%	1.03%	0.86%	1.22%	6.07%	5.17%	4.38%	5.13%	11.91%	9.15%	8.84%	10.37%

[a]Outliers identified through Cochran's test.

GM levels were removed as outliers through Cochran's test (Table 4).

Table 4. Summary of Validation Results for the TT51-1-Specific Method

| | expected value | | | | |
unkown samples	0.10%	0.50%	1.00%	5.00%	10.00%
laboratories having returned results	6	6	6	6	6
samples per laboratory	4	4	4	4	4
number of outlies	0	0	2	1	1
reason for exclusion			C. test	C. test	C. test
mean value	0.10%	0.51%	1.06%[a]	4.83%	9.62%
relative repeatability standard deviation, RSD_r	20.84%	19.96%	17.18%	24.71%	15.16%
repeatability standard deviation S_r	0.02%	0.10%	0.18%	1.19%	1.46%
relative reproducibility standard deviation RSD_R	24.50%	21.24%	18.02%	24.18%	17.63%
reproducibility standard deviation S_R	0.02%	0.11%	0.19%	1.17%	1.70%
bias (absolute value)	0.001%	0.014%	0.059%	−0.174%	−0.377%
bias	0.69%	2.85%	5.87%	−3.49%	−3.77%

Trueness. The average of four replicates of each GM level was calculated for six laboratories, respectively. The trueness of the TT51-1 method was estimated using the bias of each blind sample. As shown in Figure 2, the relative deviations from the true value for blind samples were mainly positive for most participants, ranging from −21.5% to 30.11%. After removal of identified outliers for the 1.0%, 5.0%, and 10.0% samples, the mean quantities and bias of the six participants for samples S1−S5 were calculated. The mean test values for S1, S2, S3, S4, and S5 corresponded to 0.1%, 0.51%, 1.06%, 4.83%, and 9.62%, respectively. The quantitative values deviated slightly from the true values for all tested samples with bias (%) ranging from −3.77% to 5.87%. The measure of trueness is usually expressed in terms of bias.[9] The mean bias was below 10% for each sample, with the highest bias value of 5.87% at the 1% level (S4, Table 4). According to the ENGL method acceptance criterion, the trueness should be within ±25% across the whole dynamic range. The quantitative data indicated that the TT51-1-specific method had a very credible trueness.

Repeatability and Reproducibility. The relative repeatability standard deviation (RSD_r) describing the intralaboratory variation and the relative reproducibility standard deviation (RSD_R) describing the interlaboratory variation were estimated for each sample according to ISO5725-2 (Table 4).[9] The RSD_r values for samples S1, S2, S3, S4, and S5 were 20.84%, 19.96%, 17.18%, 24.71%, and 15.16%, respectively; all RSD values were below 25%. The RSD_R values were within the range of 17.63% to 24.5%, all below 33% across the entire dynamic range. The repeatability and reproducibility satisfied the method acceptance criteria and performance requirement,[11] suggesting that the TT51-1-specific method is stable and reliable for TT51-1 quantification. The analytical results indicated that the established TT51-1 event-specific real-time PCR system can be used by different laboratories to produce acceptably accurate, reproducible, and comparable results for a given analyte.

Measurement Uncertainty of the Tested Results. The measurement uncertainty (MU) of the quantitative results was estimated according to the guidance document of CRL-GMFF.[32] The absolute standard uncertainty (u_0) and relative standard
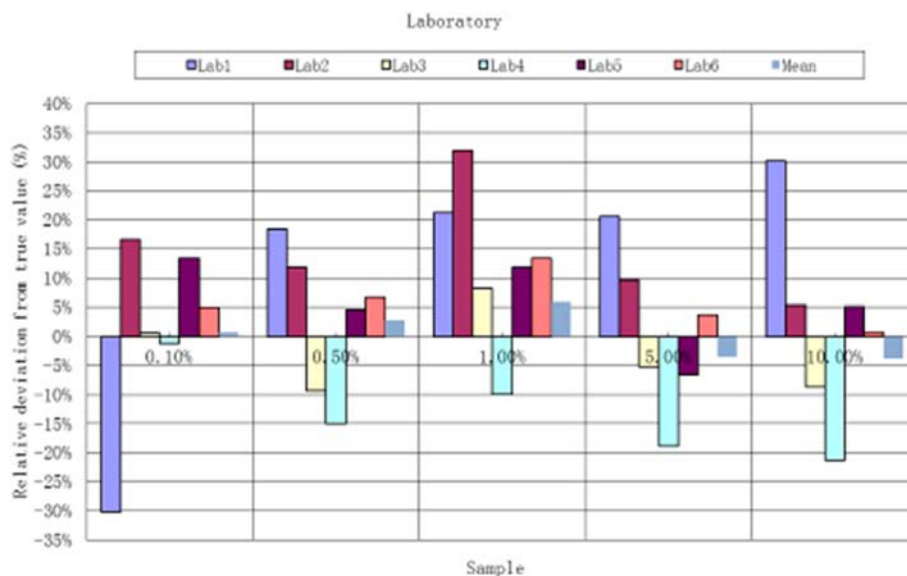
Figure 2. Relative deviation from the true value of TT51-1 test samples for all participating laboratories.

uncertainty (RSU) can be estimated by plotting the s_R values (reproducibility standard deviation) of the collaborative trial against the mean quantities of the blind samples tested (c) and calculating the linear regression (Figure 3). The value of u_0 is a
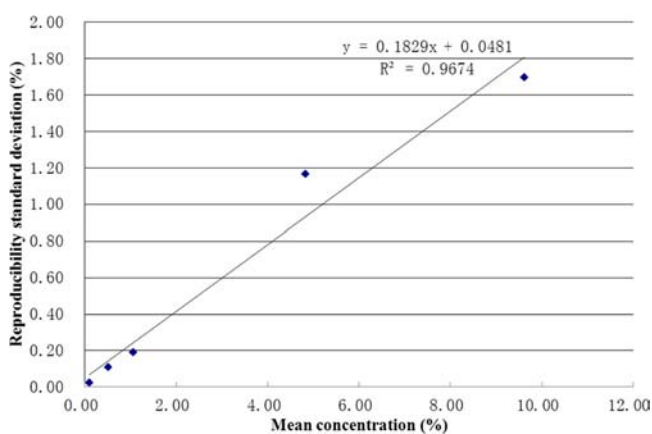
Figure 3. Linear regression produced by plotting mean measurement concentration (c) against reproducibility standard deviation (S_R).

constant, equal to the intercept of the linear regression ($u_0 = 0.0481$), and the value of RSU is equal to the slope of the linear regression (RSU = 0.1829). The critical value (LC = $2 \times u_0$) is equal to a measurement result of 0.1% TT51-1, meaning that the target TT51-1 is absent in the tested sample with 95% confidence if the estimated value is below 0.1%. The standard uncertainty (u) associated with a measurement result c is computed by the formula $u = (0.0481^2 + (0.1829 \times c)^2)^{1/2}$. The measurement uncertainty reported with tested results is usually an expanded uncertainty ($U = 2 \times (0.0481^2 + (0.1829 \times c)^2)^{1/2}$), calculated from a standard uncertainty using a coverage factor of 2. It is equivalent to a confidence level of approximately 95%. For the blind samples S1−S5, the c values of the measurement results were 0.10%, 0.51%, 1.06%, 4.83%, and 9.62% (w/w). The U values of the expanded uncertainty were calculated to be 0.10%, 0.20%, 0.40%, 1.76%, and 3.52% (w/w) for the tested samples. Therefore, the measurement concentrations should be shown as (0.10 ± 0.10)% for sample S1, (0.51 ± 0.20)% for S2,

(1.06 ± 0.40)% for S3, (4.83 ± 1.76)% for S4, and (9.62 ± 3.52)% for S5.

LOD and LOQ. According to the calculation method provided by the JRC-IRMM of the European Commission,[32] the LOD and LOQ of the TT51-1 method were calculated on the basis of the values of u_0 (0.0481) and RSU (0.1829) with the following formulas: LOD = $4u_0/(1 − 4RSU^2)$; LOQ = $(u_0^2/(RSU_{MAX}^2 − RSU^2))^{1/2}$. The LOD for the TT51-1 method was equivalent to a concentration of 0.22% TT51-1 (w/w), corresponding to 470 copies of TT51-1 target sequence in 100 ng of total rice genome DNA. In the equation for the LOQ calculation, RSU_{MAX} represents the largest acceptable relative standard uncertainty, and in the collaborative trial, the value of RSU_{MAX} refers to the greatest acceptable RSD_R value (33%). The LOQ of the TT51-1 method was estimated to be 0.18%. In the collaborative trial of the TT51-1 method, the value of LOQ should be greater than that of LOD; thus, the value of LOQ was chosen as the value of LOD (LOQ = LOD).

■ ASSOCIATED CONTENT

⑤ Supporting Information

The Ct values of DNA samples recorded by six participating laboratories were provided in Appendix 1 as supporting material. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

GMO, genetically modified organism; LOD, limit of detection; LOQ, limit of quantification; ISO, international standard organization; IUPAC, International Union of Pure and Applied Chemistry; ENGL, European Network of GMO Laboratories; EU-RL GMFF, European Union Reference Laboratory for GM Food and Feed; ISAAA, International Service for the Acquisition of Agri-Biotech Applications; NOS, nopaline synthase; FAM, 6-carboxyfluorescein; BHQ, black hole quencher; JRC-IRMM, European Commission Joint Research Center, Institute for Reference Materials and Measurements; RSD_r, relative repeatability standard deviation; RSD_R, relative reproducibility standard deviation; MU, measurement uncertainty; u_0, absolute standard uncertainty; RSU, relative standard uncertainty; s_R, reproducibility standard deviation; u, standard uncertainty; U, expanded uncertainty

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