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VERIFICATION OF REAL-TIME PCR METHODS FOR QUALITATIVE AND QUANTITATIVE TESTING OF GENETICALLY MODIFIED ORGANISMS

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ABSTRACT

This article reports the experience of a university laboratory in accreditation of molecular biology methods for genetically modified (GM) organisms detection according to the International Organization for Standardization (ISO)/International Electrotechnical Commission 17025 standard. Verification studies were performed for one screening (35S promoter), one construct-specific (p35S/Chloroplast Transit Peptides-5-enolpyruvylshikimate-3-phosphate synthase [CTP-EPSPS]) and one event-specific (Bt11 maize) real-time polymerase chain reaction methods. Our procedures were performed as described in the ISO 21570 2005, except for the analysis done through a DNA Engine Opticon 2 MJ research instrument. The following method–performance characteristics were evaluated: amplification efficiency, linearity, dynamic range, limit of detection, limit of quantification, precision and trueness. Comparison of the performance results obtained for quantitative methods with the values of inter-laboratory ring trials, coordinated by the European Union Reference Laboratory for GM Food and Feed, confirmed that the laboratory is able to fulfill the published criteria.

PRACTICAL APPLICATIONS

This article illustrates the experience of a university laboratory with the International Organization for Standardization/International Electrotechnical Commission 17025 accreditation for genetically modified organisms testing. In-house verification has been carried out for one screening (35S promoter), one construct-specific (p35S/Chloroplast Transit Peptides-5-enolpyruvylshikimate-3-phosphate synthase [CTP-EPSPS]) and one event-specific (Bt11 maize) real-time polymerase chain reaction methods. We reported for the first time validation data obtained through a DNA Engine Opticon 2 MJ Research instrument. The described experimental procedure could represent a pattern for researchers interested in setting up verification of validated methods for genetically modified organism quantitative and qualitative testing.

INTRODUCTION

The use of genetically modified organisms (GMOs) and GMO-derived products in food and feed is subject to regulations that differ from one country to another. In the European Union (EU), the food labeling is mandatory when more than 0.9% of the food ingredients, considered individually, are of GM origin (EC regulation 1829/2003; Taverniers *et al.* 2005; Elenis *et al.* 2008). The implementation of labeling regulations is based on the reliability and precision of techniques for GMO detection. DNA is widely used as the target for GMO analysis because of its stability and high detectability in processed matrixes and real-time polymerase chain reaction (PCR) technique is the method of choice used routinely in food control laboratories as it combines high sensitivity with the possibility to quantify

the GMO content (Holst-Jensen and Berdal 2004; Chaouachi *et al*. 2007). The GMO detection strategy usually includes an initial screening for the mostly used genetic elements, including the 35S promoter of cauliflower mosaic virus and the nopaline synthase terminator of *Agrobacterium tumefaciens*. On the basis of the screening results, the construct- and/or event-specific elements are addressed to identify and quantify a specific GM event (Hohne *et al*. 2002; Gaudron *et al*. 2009; Del Gaudio *et al*. 2010). The amount of the transgenic target and the reference plant gene are determined by interpolation with a standard curve and the result is provided as the percentage of GMO in the sample tested (Von Gotz 2010).

The reference methods for the detection and quantification of transgenic crops in the EU are provided by applicants for authorization and validated by the EU Reference Laboratory for GM Food and Feed (EURL-GMFF), assisted by the European Network of GMO Laboratories (ENGL). In recent years, the International Organization for Standardization (ISO) 21569 2005 and 21570 2005, including some of the validated methods mentioned earlier, were made available for laboratories accredited according to the ISO/International Electrotechnical Commission (IEC) 17025 2005. Before a new method is introduced, the laboratory has to verify that it is able to achieve the performance characteristics of the method as published in the inter-laboratory validation report. As extensively reported in Zel *et al*. (2008), a series of parameters have to be tested to verify the agreement with the ENGL method–acceptance criteria (2008):

- amplification efficiency: the average value of the slope of the standard curve should be in the range of ($-3.1 \geq$ slope ≥ -3.6), corresponding to an efficiency between 90 and 110%;
- R^2 coefficient: the average value of the squared correlation coefficient (R^2) of the standard curve obtained by linear regression analysis should be ≥ 0.98;
- dynamic range: the range of concentrations over which the method performs in a linear manner with an acceptable level of trueness and precision should include the 1/10 and at least 5 times the target concentration (0.09% and 4.5% for a 0.9% GMO concentration);
- limit of detection (LOD): the minimum level at which the analyte can reliably be detected should be less than 1/20th of the target concentration (LOD < 0.045% for a 0.9% GMO concentration);
- limit of quantification (LOQ): the minimum level at which the analyte can reliably be quantified should be less than $1/10^{th}$ of the target concentration (LOQ < 0.09% for a 0.9% GMO concentration);
- precision: the relative repeatability standard deviation should be below 25% over the entire dynamic range of the method;
- trueness: the bias between mean measured value and accepted reference value should be within $\pm 25\%$ of the accepted reference value across the entire dynamic range of the method.

This article illustrates the experience of a university laboratory with the ISO/IEC 17025 accreditation for GMO testing. In-house verification has been carried out for one screening (35S promoter), one construct-specific (p35S/Chloroplast Transit Peptides-5-enolpyruvylshikimate-3-phosphate synthase [CTP-EPSPS]) and one event-specific (Bt11 maize) real-time PCR method.

In summary, the described experimental procedure could be a representative pattern for accredited laboratories interested in implementation of validated methods for GMO quantitative and qualitative testing.

MATERIALS AND METHODS

Samples

Powdered certified reference materials (CRM) containing 0, 0.1, 1, 5% Bt11 maize (ERM-BF412) and GTS 40-3-2 soy (ERM-BF410), respectively, prepared by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) were obtained commercially (Sigma-Aldrich, Seelze, Germany). Test materials from the GeMMA proficiency tests were from Food Analysis Performance Assessment Scheme (FAPAS) (Central Science Laboratory, Sand Hutton, UK).

DNA Extraction

The cetyltrimethylammonium bromide DNA extraction protocol was used in accordance with the UNI EN ISO 21571 2005. All extractions were made in duplicate and the DNA concentrations were measured through the Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

TaqMan Probes and Primers

The sequence of primers and TaqMan probes were used in this study as suggested in the UNI EN ISO 21570 2005 (Table 1). All primers and probes were supplied by Eurofins MWG Operon (Ebersberg, Germany).

Real-Time PCR

The real-time PCR reactions were performed on a DNA Engine Opticon 2 MJ research (Biorad, Hercules, CA). Five micro liters of DNA were amplified in a total volume of 25 μL containing 1X TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) and the thermal

TABLE 1. LIST OF PRIMERS AND TAQMAN PROBES

Target	Primer and probe name	Sequence	Conc. (nM)	Amplicon size (bp)
adh1	ADHFF3	5′- CGTCGTTTCCCATCTCTTCCTCC - 3′	300	134
	ADHRR4	5′- CCACTCCGAGACCCTCAGTC - 3′	300	
	ADH1-MDO	5′ - FAM - AATCAGGGCTCATTTTCTCGCTCCTCA - TAMRA – 3′	200	
le1	GM1-F	5′ - CCAGCTTCGCCGCTTCCTTC - 3′	600	74
	GM1-R	5′ - GAAGGCAAGCCCATC TGCAAGCC - 3′	600	
	Probe GM1	5′ - FAM - CTTCACCTTCTATGCCCCTGACAC - TAMRA - 3′	120	
p35S	35S-F	5′- GCCTCTGCCGACAGTGGT - 3′	300	82
	35S-R	5′- AAGACGTGGTTGGAACGTCTTC - 3′	900	
	35S-TMP	5′ - FAM - CAAAGATGGACCCCCACCCAC - TAMRA - 3′	100	
GTS 40-3-2 construct	RR1-F	5′- CATTTGGAGAGGACACGCTGA - 3′	600	74
	RR1-R	5′- GAGCCATGTTGTTAATTTGTGCC - 3′	600	
	Probe RR1	5′ - FAM - CAAGCTGACTCTAGCAGATCTTTC - TAMRA - 3′	120	
Bt11 event	Bt113JFor	5′- GCGGAACCCCTATTTGTTTA - 3′	750	70
	Bt113JRev	5′- TCCAAGAATCCCTCCATGAG - 3′	750	
	Bt113JFT	5′ - FAM - AAATACATTCAAATATGTATCCGCTCA - TAMRA - 3′	250	

From left to right target, name, sequence, concentration used and amplicon size are shown.

cycling conditions were as follows: 2 min of Uracil-N-glycosylase (UNG) incubation at 50C, 10 min of denaturation at 95C followed by 45 cycles of a two-step program (denaturation at 95C for 15 s and annealing/extension at 60C for 1 min). Fluorescence threshold and baseline setting was adjusted with the aid of the Opticon Monitor software (Biorad).

Data Collection and Statistical Analysis

Calibration curves were constructed by diluting the DNA extracted from 5% Bt11 CRM in order to contain 258, 129, 65, 16 and 5 ng of DNA corresponding to 4734, 2366, 1184, 296, 98 copies of the transgenic sequence and 94676, 47340, 23680, 5920, 1960 maize genome copies per reaction. DNA extracted from 5% GTS 40-3-2 CRM was diluted in order to contain 100, 50, 12.5, 4.2 and 1.4 ng of DNA corresponding to 4425, 2212, 553, 184, 61 copies of the transgenic sequence and 88495, 44248, 11062, 3687, 1229 soy genome copies per reaction. Standard curves were constructed by plotting Ct values against $\log_{10}$ of DNA amount and fitted by linear least square regression. The PCR efficiency was calculated according to the following formula: $10^{(-1/\text{slope})} - 1$. For determination of the precision of quantitative methods, maize and soy CRM at 0.1, 1 and 5% GMO content were analyzed in repeated reactions carried out by the same operator within a short period (1 week). Dilutions of standards and test samples were run in quadruplicate. Standard deviations and coefficients of variation were calculated for the Ct values of replicated measurements. Copy numbers of transgenic and taxon-specific targets were calculated by interpolation and their ratio was expressed as percentage to determine the transgenic content. Mean quantities, repeatability standard deviations (RSD) and repeatability relative standard deviations (RSDr) were determined for 0.1, 1 and 5% CRM samples. The uncertainty (u_m) was calculated by: RSD/ $\checkmark n$, where n was the number of PCR runs. The combined uncertainty u_Δ was given by: $u_\Delta = \sqrt{u_m^2} + \sqrt{u_{CRM}^2}$, where u_{CRM} was uncertainty of CRM reported on IRMM certificate. Finally, expanded uncertainty (U_Δ) was calculated as: $t \times u_\Delta$ where t was the Student factor ($t = 2.262$ for $n = 10$, $t = 2.306$ for $n = 9$ and $t = 2.365$ for $n = 8$ at $P = 95\%$). To test the sensitivity of methods, serial DNA dilutions of the DNA extracted from 5% CRM from high to very low copy number of the target were analyzed in quadruplicate in two independent PCR runs. For LOD and LOQ determinations, we proceeded by calculating the lowest copy number corresponding to an RSDr $\leq$ 33% for LOD and $\leq$ 25% for LOQ. Finally, we estimated the trueness by using the measures of bias between the mean measured value and the reference value of CRM (reported on IRMM certificate) at 0.1, 1 and 5% GMO content. Relative bias was expressed as the ratio between the absolute bias and the reference value at each GMO level.

RESULTS AND DISCUSSION

In this study, we performed a verification procedure of a real-time PCR method for qualitative detection of 35S promoter and two quantitative methods targeting, respectively, the construct (p35S/ CTP-EPSPS) specific for GTS 40-3-2 soy and the maize event Bt11-specific sequence. The procedure involves the amplification of soy-specific lectin (le1) and maize-specific alcohol dehydrogenase (adh1) reference genes. Previous validation studies concerning EURL-GMFF developed methods carried out with Applied Biosystems

TABLE 2. SLOPE, EFFICIENCY, SQUARED COEFFICIENT OF CORRELATION (R^2), DYNAMIC RANGE, LOD AND LOQ FOR TESTED ASSAYS

Assay	Slope	Efficiency (%)	R^2	Dynamic range (copies)	LOD (copies)	LOQ (copies)
adh1	–3.40	96.8	0.996	94,676–49	4	49
le1	–3.37	98.0	0.997	88,495–43	10	43
p35S	–3.70	86.6	0.992	4,425–198	114	198
GTS 40-3-2 construct	–3.32	100.0	0.992	4,425–39	12	39
Bt11 event	–3.32	100.0	0.994	4,700–26	5	26

LOD, limit of detection; LOQ, limit of quantification.

(Hubner *et al.* 2001; Ronning *et al.* 2003), Bio-Rad i-Cycler (Scholtens *et al.* 2010) or capillary Light Cycler real-time PCR instrument (Sieradzki and Kwiatek 2009). This article reports for the first time validation data obtained through a DNA Engine Opticon 2 MJ Research instrument.

The primers and probes were the same as published in the standard UNI EN ISO 21570 2005. Primer and probe concentrations were unchanged with respect to the reference protocol and the TaqMan Universal PCR Master Mix was used for all the assays in order to minimize the optimization procedures. Before real-time amplification, the quality and amount of DNA were assessed spectrophotometrically (data not shown). Because the methods were previously validated by EURL-GMFF, we did not evaluate some parameters as applicability, practicability and specificity (Zel *et al.* 2008).

The first step of verification consisted in verifying that all the real-time PCR assays showed an acceptable efficiency and linearity, in agreement with the requirements of the ENGL (2008). The values in Table 2 are the mean of nine (le1 and GTS 40-3-2) and 10 (adh1and Bt11) repeated PCR runs, while for the p35 screening method, three PCR reactions were performed. The average slope of the regression line should be within −3.1 and −3.6; all the slopes met the ENGL criteria (2008), reaching the ideal value of −3.32 for le1 and Bt11 assays. Consequently, the average reaction efficiencies ranged between 96.8 and 100%. Only the slope for p35S was −3.70, corresponding to an efficiency of 86.6%. The linearity of reaction was very high, as R^2 coefficient was over 0.992 for all the tested assays. The dynamic range of all methods was limited by the amount of target DNA copies isolated from 5% CRM at the higher value and by the LOQ at low-range value (Hubner *et al.* 2001; Ronning *et al.* 2003; Sieradzki and Kwiatek 2009; Scholtens *et al.* 2010). As shown in Table 2, this range expanded to four orders of magnitude for reference gene assays (adh1 and le1) and to three orders of magnitude for GM assays (GTS 40-3-2 soy and Bt11 maize). The p35S method worked in a linear manner with an acceptable precision in a limited range of concentrations.

The LOD and LOQ are defined as the lowest content that can be detected and can be measured with reasonable statistical certainty, at least 95% probability, respectively. Our results met the acceptance criteria (maximum 25 copies for LOD and 50 copies for LOQ) for soy and maize quantitative assays required from ENGL (2008, 2011). For GTS 40-3-2 quantification method the corresponding relative LOD was 0.015% and relative LOQ was 0.046%. Previous validation by EURL-GMFF reported a 0.06% as relative LOQ (ISO 21570 2005). Similarly, Hubner *et al.* (2001) reported a 0.01% as relative LOD and a 0.06% as relative LOQ, while Sieradzki and Kwiatek (2009) found that LOD was 0.025% and LOQ was 0.075%, although using alternative construct-specific primers and TaqMan probes. As regards Bt11 quantification method, the relative LOD was 0.015% and the relative LOQ was 0.07%. This means that we reached a sensitivity higher than EURL-GMFF, which made the validation of Bt11 event-specific assay coupled with adh1 reference gene reporting a 0.1% as relative LOD and LOQ (ISO 21570 2005). Other authors determined relative LOQ at 0.05% (Ronning *et al.* 2003) and 0.06% (Sieradzki and Kwiatek 2009) with maize invertase being selected as reference gene. Instead for p35S screening LOD and LOQ were, respectively, 114 and 198 copies, corresponding to high-relative LOD (0.14%) and LOQ (0.24%). Major efforts will be needed to improve the sensitivity of this assay.

The precision of the tested quantitative methods was expressed in terms of RSDr value, which is the ratio of the standard deviation of the mean divided by the mean value of measurements. We calculated the precision by repeated measurements at three distinct levels of GMO, i.e., 0.1, 1 and 5%. RSDr value should be below 25% over the entire dynamic range. As can be seen in Table 3, our results complied with ENGL criteria (2008, 2011) except for the RSDr value relative to 0.1% Bt11 maize (35.7%). Similar result was obtained by EURL-GMFF whose RSDr at the 0.1% Bt11 level was 33.5% (ISO 21570 2005). For GTS 40-3-2 assay, we reached better values of precision than those reported by EURL-GMFF in ISO 21570 2005 (from 10 to 33%), as our RSDr ranged between 8.52 and 22.2%. Therefore these results were very close to the precision values reported by Hubner *et al.* (2001) and Sieradzki and Kwiatek (2009) for their method.

Expanded uncertainty was below 25% of the mean measured value as required by ENGL criteria (2008, 2011) except for 0.1% GTS 40-3-2 (66%) and 0.1% Bt11 (35%).

TABLE 3. DATA ON PRECISION AND TRUENESS OF GTS 40-3-2 SOY AND BT11 REAL-TIME QUANTITATIVE METHODS

	GTS 40-3-2 soy			Bt11 maize		
Certified value (%)	0.10	1.00	5.00	0.12	0.98	4.89
N. Measures	9	9	9	8	10	10
Mean value (%)	0.09	1.13	4.81	0.14	1.12	5.0
Repeatability standard deviation (%)	0.02	0.18	0.41	0.05	0.14	0.37
Repeatability relative standard deviation (%)	22.2	15.9	8.52	35.7	12.5	7.4
Expanded uncertainty (%) (confidence level 95%)	0.06	0.23	0.68	0.05	0.14	0.35
Bias (absolute value)	–0.01	0.13	–0.19	0.02	0.14	0.11
Bias (%)	–10.0	13.0	–3.8	16.6	14.3	2.2

This result could be explained with the contribution of CRM uncertainty, as reported on the IRMM certificate, which has a considerable weight on the final value of expanded uncertainty at this level of GMO content.

Finally, we determined the trueness by measuring the bias at 0.1, 1 and 5% GMO level. The expanded uncertainty was larger than the difference between measured and certified mean value. This means that the measured mean value was not significantly different from the certified value and then the tested methods had no bias. According to the ENGL method performance requirements, trueness should be within $\pm$25% across the entire dynamic range. In this case, both the tested methods satisfied these requirements, because the highest values of relative bias were 13% at 1% level GM soy and 16.6% at 0.12% Bt11 maize.

It is a requirement for accreditation to ISO/IEC 17025 that the laboratory takes part to a proficiency testing scheme. Our laboratory participated to the GeMMA proficiency test coordinated by FAPAS, obtaining satisfactory performance results. In fact, z score value of 1.0 for GTS 40-3-2 soy (GeM SU33 round) and a z score of 0.4 for BT11 maize (GeM SU09 round) have been obtained, included in the accepted range ($|z| \leq 2$).

CONCLUSIONS

A verification study has been carried out for one screening (35S promoter), one construct-specific (p35S/ CTP-EPSPS) and one event-specific (Bt11 maize) real-time PCR methods. We optimized these PCR assays, originally developed for an Applied Biosystems instrument, on a DNA Engine Opticon 2 MJ Research thermocycler. The method performance has been evaluated with respect to the ENGL criteria and our results were compared with those reported for inter-laboratory ring-trial carried out by the EURL-GMFF and to literature available studies. While the screening method requires an additional optimization work, satisfying performance characteristics allowed the applicability of tested quantitative methods for GMO analysis in our accredited ISO/IEC 17025 laboratory. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

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REFERENCES

CHAOUACHI, M., GIANCOLA, S., ROMANIUK, M., LAVAL, V., BERTHEAU, Y. and BRUNEL, D. 2007. A strategy for designing multi-taxa specific reference gene systems. Example of application-ppi phosphofructokinase (ppi-PPF) used for the detection and quantification of three taxa: Maize (*Zea mays*), cotton (*Gossypium hirsutum*) and rice (*Oryza sativa*). J. Agric. Food Chem. *55*, 8003–8010.

del GAUDIO, S., CIRILLO, A., DI BERNARDO, G., GALDERISI, U. and CIPOLLARO, M. 2010. A preamplification approach to GMO detection in processed foods. Anal. Bioanal. Chem. *396*, 2135–2142.

ELENIS, D.S., KALOGIANNI, D.P., GLYNOU, K., IOANNOU, P.C. and CHRISTOPOULOS, T.K. 2008. Advances in molecular techniques for the detection and quantification of genetically modified organisms. Anal. Bioanal. Chem. *392*, 347–354.

EUROPEAN NETWORK OF GMO LABORATORIES (ENGL). 2008. Definition of minimum performance requirements for analytical methods of GMO testing.

EUROPEAN NETWORK OF GMO LABORATORIES (ENGL) 2011. Verification of analytical methods for GMO testing when implementing interlaboratory validated methods.

GAUDRON, T., PETERS, C., BOLAND, E., STEINMETZ, A. and MORIS, G. 2009. Development of a quadruplex-real-time-PCR for screening food for genetically modified organism. Eur. Food Res. Technol. *229*, 295–305.

HOHNE, M., SANTISI, C.R. and MEYER, R. 2002. Real-time multiplex PCR: An accurate method for the detection and quantification of 35SCaMV promoter in genetically modified maize-containing food. Eur. Food Res. Technol. *215*, 59–64.

HOLST-JENSEN, A. and BERDAL, K.G. 2004. The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds. J. AOAC Int. *87*, 927–936.

HUBNER, P., WAIBLINGER, H.U., PIETSCH, K. and BRODMANN, P. 2001. Validation of PCR methods for quantitation of genetically modified plants in food. J. AOAC Int. *84*, 1855–1864.

ISO 21569 2005. Foodstuffs-Methods of analysis for the detection of genetically modified organisms and derived products-Qualitative nucleic acid based methods. Geneva, Switzerland.

ISO 21570 2005. Foodstuffs-Methods of analysis for the detection of genetically modified organisms and derived products-Quantitative nucleic acid based methods. Geneva, Switzerland.

ISO/IEC 17025 2005. General requirements for the competence of testing and calibration laboratories Geneva, Switzerland.

REGULATION (EC). 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Off. J. Eur. Commun. 1–23.

RONNING, S.B., VAITILINGOM, M., BERDAL, K.G. and HOLST-JENSEN, A. 2003. Event specific quantitative PCR for genetically-modified Bt11 maize (*Zea mays*). Eur. Food Res. Technol. *216*, 347–354.

SCHOLTENS, I.M., KOK, E.J., HOUGS, L., MOLENAAR, B., THISSEN, J.T. and VAN DER VOET, H. 2010. Increased efficacy for in-house validation of real-time PCR GMO detection methods. Anal. Bioanal. Chem. *396*, 2213–2227.

SIERADZKI, Z. and KWIATEK, K. 2009. Validation of real-time PCR methods for the quantification of genetically-modified maize and soybean. Bull. Vet. Inst. Pulawy *53*, 741–746.

TAVERNIERS, I., WINDELS, P., VAÏTILINGOM, M., MILCAMPS, A., VAN BOCKSTAELE, E., VAN DEN EEDE, G. and DE LOOSE, M. 2005. Event-specific plasmid standards and real-time PCR methods for transgenic Bt11, Bt176, and GA21 maize and transgenic GT73 canola. J. Agric. Food Chem. *53*, 3041–3052.

VON GOTZ, F. 2010. See what you eat – Broad GMO screening with microarrays. Anal. Bioanal. Chem. *396*, 1961–1967.

ZEL, J., MAZZARA, M., SAVINI, C., CORDEIL, S., CAMLOH, M., STEBIH, D., CANKAR, K., GRUDEN, K., MORISSET, D. and VAN DEN EEDE, G. 2008. Method validation and quality management in the flexible scope of accreditation: An example of laboratories Testing for genetically modified organisms. Food Anal. Methods *1*, 61–72.