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Compendium of reference methods for GMO analysis

European Union Reference Laboratory for GM Food and Feed (EURL-GMFF)
European Network of GMO Laboratories (ENGL)

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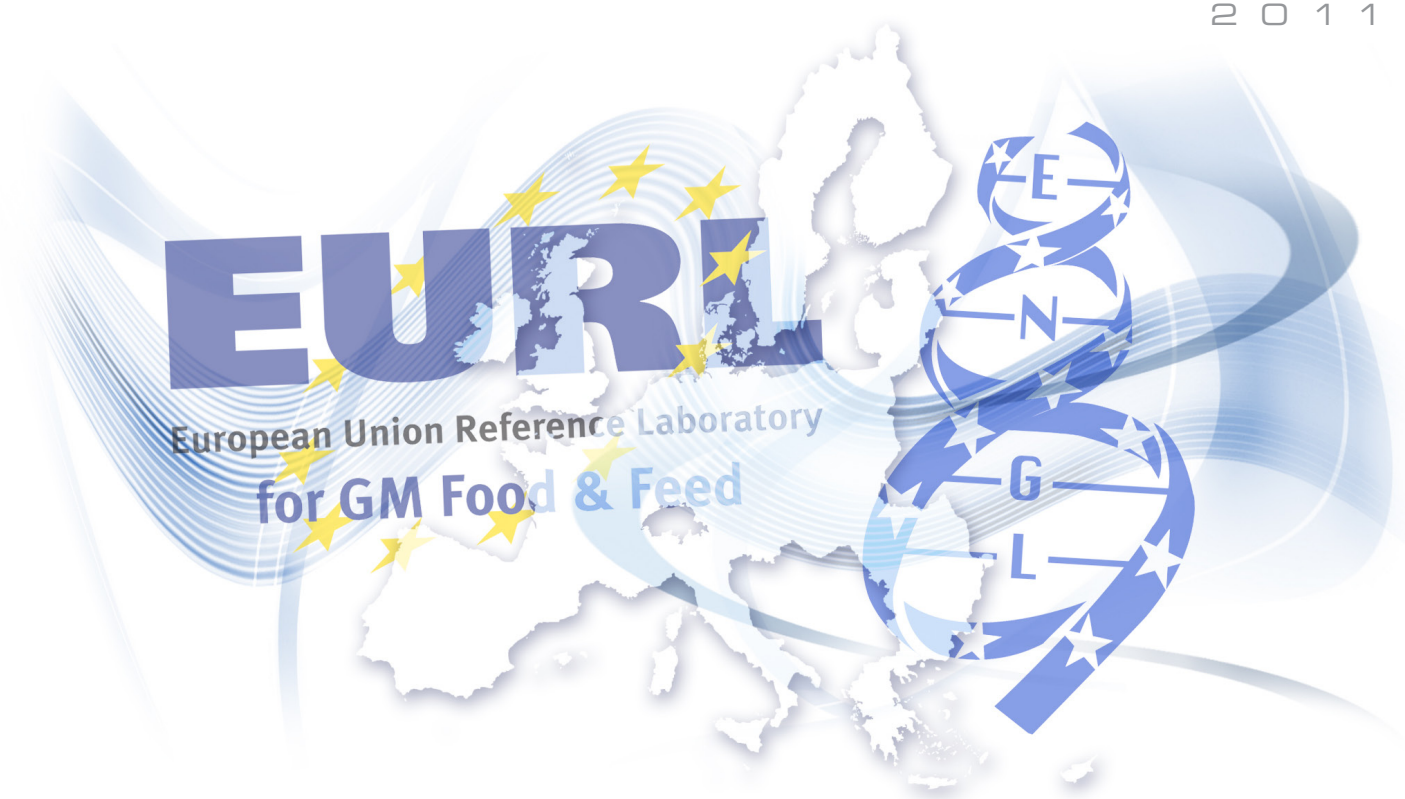

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This report has been produced by the Joint Research Centre as European Union Reference Laboratory for GM Food and Feed, in collaboration with the European Network of GMO Laboratories. It aims at providing a list of reference methods for GMO analysis that have been validated in a collaborative trial according to the principles and requirements of ISO 5725 and/or IUPAC protocol. All efforts have been made to ensure that the information provided is accurate and correct but no responsibility can be taken for the data and information reported in the method validation publication.

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Preface

This compendium of validated methods was assembled by the Molecular Biology and Genomics Unit of the Joint Research Centre's Institute for Health and Consumer Protection, nominated "European Union Reference Laboratory for Genetically Modified Food and Feed", in collaboration with the European Network of GMO Laboratories (ENGL).

I am very grateful to the following colleagues (listed in alphabetical order) who have collaborated in its preparation:

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Executive Summary

The legal mandate of the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) regarding GMO analysis is laid down in Regulation (EC) No 1829/2003 on "genetically modified food and feed" and Regulation (EC) No 882/2004 "official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules".

The EURL-GMFF is supported by the European Network of GMO Laboratories (ENGL). This network is formed by almost 100 national enforcement laboratories and provides a unique forum of European scientific expertise on GMO analysis.

In accordance with article 32(1) of Regulation (EC) No 882/2004, the European Union Reference laboratories for feed and food are responsible, amongst others, for "providing national reference laboratories with details of analytical methods, including reference methods".

In this frame, this "Compendium of Reference Methods for GMO Analysis" has been produced jointly by the EURL-GMFF and the ENGL.

This "Compendium of Reference Methods for GMO Analysis" aims at providing a technical state of the art of the detection methods applied in GMO analysis that have been validated according to international standards.

Since the concept of "reference methods" per se is not strictly defined in EU legislation on GMOs, the following selection criteria were applied to decide on inclusion of methods in the present "Compendium of Reference Methods for GMO Analysis":

- Considering the largest common denominator in the global framework of methodology applied in GMO analysis, this first issue of the "Compendium on Reference Methods for GMO Analysis" (2010 edition) is focused on Polymerase Chain Reaction (PCR) methods i.e. DNA-based detection methods. In follow-up editions, it is foreseen to extend the scope of this "Compendium of Reference Methods for GMO Analysis" to include also DNA extraction methods and protein-based detection methods.

- The methods collected in the current Compendium have been selected based on their reported compliance with ISO 5725 international standard [Accuracy (trueness and precision) of measurement methods and result] and/or the IUPAC (International Union of Pure and Applied Chemistry) "Protocol for the design, conduct and interpretation of method performance studies" (Horwitz, W., *Pure & Appl. Chem.*, 67, 331-343, 1995).

In short, all methods included in the current Compendium (2010 edition) are therefore DNA-based detection methods which have been validated through a collaborative trial according to ISO 5725 and/or the IUPAC protocol.

Once selected according to the above criteria, a list of 79 reference methods has been compiled. Each method has then been described in a comprehensive summary which provides the essential information related to the validated method. Not all details are given for each method but all necessary references are provided for further information about each method.

1. Introduction

The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) is hosted by the European Commission Joint Research Centre in Ispra (Italy).

The legal mandate of the EURL-GMFF regarding GMO analysis is laid down in Regulation (EC) No 1829/2003 on "Genetically modified food and feed" and Regulation (EC) No 882/2004 "Official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules" (see further details below). Within this legal framework, a core activity of the EURL-GMFF is the validation of GMO detection methods as an integral part of the EU regulatory approval process for GMOs.

The EURL-GMFF is supported by the European Network of GMO Laboratories (ENGL). This network is formed by almost 100 national enforcement laboratories and provides a unique forum of European scientific expertise on GMO analysis.

In accordance with article 32(1) of regulation (EC) No 882/2004, the European Union reference laboratories for feed and food are responsible, amongst others, for "providing national reference laboratories with details of analytical methods, including reference methods".

In this frame, this "Compendium of Reference Methods for GMO Analysis" has been produced jointly by EURL-GMFF and the ENGL.

The decision to proceed with this joint publication between EURL-GMFF and ENGL was taken through a unanimous electronic vote from ENGL members in April 2010.

2. Legal Background

Since the early 1990s the European Union (EU) has established an extensive legal framework on GMOs.

A key objective of the EU legislation on GMOs is to protect human and animal health as well as the environment: a genetically modified organism (GMO) or a food or feed product derived from a GMO can only be placed on the EU market after it has been authorized, on the basis of a stringent approval procedure, based on a EU scientific assessment of the risks to health and the environment.

The EU legislation on GMOs also aims to provide information to EU consumers through mandatory GM labeling of food and feed products containing, consisting of or produced from GMOs.

In this frame, GMO analysis plays a key role in the implementation of the EU legislation on GMOs, for instance to ensure appropriate labeling of approved GMO products or to detect the possible presence of unapproved GMO products on the market.

Submission and validation of GMO detection methods are actually an integral part of the EU regulatory approval process for GMOs. Regulation (EC) No 1829/2003 on genetically modified food and feed (articles 5 and 17) provides that the application for authorisation should include, amongst others:

- Methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food and/or in foods produced from it.

- Samples of the food and their control samples, and information as to the place where the reference material can be accessed. Control samples mean the GMO or its genetic material (positive sample) and the parental organism or its genetic material that has been used for the genetic modification (negative sample).

Article 32 of Regulation (EC) No 1829/2003 provides that the EU Reference Laboratory for GM Food and Feed and its duties are those referred in the Annex of Regulation (EC) No 1829/2003. The Annex of Regulation (EC) No 1829/2003 (as amended by Annex III of Regulation (EC) No 1981/2006) provides that:

1. The EU reference laboratory referred to in Article 32 is the Commission's Joint Research Centre.

2. For the duties and tasks outlined in this Annex, the EU reference laboratory shall be assisted by the national reference laboratories referred to in Article 32, which shall consequently be considered as members of the consortium referred to as the "European Network of GMO laboratories".

3. The EU reference laboratory shall be responsible, in particular, for:

(a) the reception, preparation, storage, maintenance and distribution to the members of the European Network of GMO laboratories of the appropriate positive and negative control samples, subject to assurance given by such members of the respect of the confidential nature of the data received where applicable;

(b) without prejudice to the responsibilities of the EU reference laboratories laid down in Article 32 of Regulation (EC) No 882/2004, the distribution to national reference laboratories within the meaning of Article 33 of that Regulation of the appropriate positive and negative control samples, subject to assurance given by such laboratories of the respect of the confidential nature of the data received where applicable;

(c) evaluating the data provided by the applicant for authorisation for placing the food or feed on the market, for the purpose of testing and validation of the method for sampling and detection;

(d) testing and validating the method for detection, including sampling and identification of

the transformation event and, where applicable, for the detection and identification of the transformation event in the food or feed;

(e) submitting full evaluation reports to the European Food Safety Authority.

Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 provides further details on the applications for authorisation of GM food and feed,

including the method(s) of detection, sampling and event specific identification of the transformation event, as provided for in articles 5(3) and 17(3) of Regulation (EC) No 1829/2003.

In particular Annex I of Regulation (EC) No 641/2004 on "method validation" provides detailed technical provisions on the type of information on detection methods that shall be provided by the applicant and that is needed to verify the preconditions for the fitness of the method. This includes information about the method as such and about the method testing carried out by the applicant.

Annex I of Regulation (EC) No 641/2004 also confirms that the validation process will be carried out by the EURL according to internationally accepted technical provisions. All guidance documents referred to in this Annex or produced by the European Union Reference Laboratory (EURL) are to be made available by the EURL.

Regulation (EC) No 1981/2006 provides further detailed rules specific for the implementation of article 32 of Regulation (EC) No 1829/2003. Annex I of Regulation (EC) No 1981/2006 lays down the minimum requirements to be fulfilled by the National Reference Laboratories assisting the EURL (including to be accredited, or being in the process of accreditation according to EN ISO/IEC 17025).

Note: detailed information on the activities of the EU Reference Laboratory for GM Food and Feed is available at http://gmo-crl.jrc.ec.europa.eu/default.htm

In addition to the tasks of the EURL provided for in Regulation (EC) No 1829/2003 concerning the validation of methods for the GM food/feed authorisation procedure, the EURL has additional responsibilities under Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

In particular, Title III of Regulation (EC) No 882/2004 deals with the responsibilities of "reference laboratories" (including EU Reference Laboratories EURL - see article 32 and National Reference Laboratories NRL - see article 33).

Pursuant to Article 32 of Regulation (EC) No 882/2004, all EU Reference Laboratories (EURLs) for feed and food referred to in Annex VII (including therefore the EU Reference Laboratory for Genetically Modified Organisms) are responsible for:

(a) providing national reference laboratories with details of analytical methods, including reference methods;

(b) coordinating application by the national reference laboratories of the methods referred to in (a), in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;

(c) coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing national reference laboratories of advances in this field;

(d) conducting initial and further training courses for the benefit of staff from national reference laboratories and of experts from developing countries;

(e) providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses;

(f) collaborating with laboratories responsible for analysing feed and food in third countries.

Pursuant to Article 33 of Regulation (EC) No 882/2004, the Member States should designate one or more National Reference Laboratories (NRLs) for each EURL referred to in article 32 (including therefore the EU Reference Laboratory for Genetically Modified Organisms). The responsibilities of these NRLs are to:

(a) collaborate with the EU reference laboratory in their area of competence;

(b) coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11;

(c) where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;

(d) ensure the dissemination to the competent authority and official national laboratories of information that the EU reference laboratory supplies;

(e) provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans adopted in accordance with Article 53;

(f) be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.

3. Accreditation

Article 12 of Regulation (EC) No 882/2004 provides that official control laboratories may only be designated by their Competent Authorities if they operate and are assessed and accredited in accordance with the following European standards:

(a) EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'

(b) EN 45002 on 'General criteria for the assessment of testing laboratories'

(c) EN 45003 on 'Calibration and testing laboratory accreditation system - General requirements for operation and recognition'

Therefore, accreditation of laboratories conducting official control, including testing for GMOs, is a regulatory requirement of the EU legislative framework on GMOs.

This concept is further confirmed by the specific regulatory requirement set out in Annex I of Regulation (EC) No 1981/2006 (on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003) which provides that "laboratories assisting the EU reference laboratory for testing and validating the method for detection must be accredited, or being in the process of accreditation according to EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories' or an equivalent international standard which ensures that the laboratories:

- have suitably qualified staff with adequate training in analytical methods used for the detection and identification of GMOs and GM food and feed,
- possess the equipment needed to carry out the analysis of GMOs and GM food and feed,
- have an adequate administrative infrastructure,
- have sufficient data-processing capacity to produce technical reports and to enable rapid communication with the other laboratories participating in the testing and validation of detection methods;

In general, quality assurance is anyway a prerequisite for accurate and reliable results in food and feed testing, EN ISO/IEC 17025 being recognized worldwide as the base standard.

Accreditation standard EN ISO/IEC 17025 specifies the criteria for selection of methods; methods that are appropriately described and published in international, regional or national standards, or by reputable technical organizations, shall be selected. In cases when a method is not considered as a "standard" method, the laboratory shall carry out the validation of such methods, with a considerable effort in terms of resources. Therefore, the availability of methods validated according to recognized international standards is a key benefit for the implementation of quality assurance systems in testing laboratories.

In order to maintain up-to-date GMO analysis, a flexible scope of accreditation is generally considered essential, both at the qualitative and the quantitative level. Due to the constantly developing number of GMOs on the market, a large number of new methods need to be readily available and introduced into the scope of accreditation of control laboratories. For this, testing laboratories may follow standard procedures as described in Zel et al. (Method Validation and Quality Management in the Flexible Scope of Accreditation: An example of Laboratories Testing for Genetically Modified Organisms, *Food Analytical Methods, Springer Science,* 2008) whenever a new procedure validated elsewhere is to be incorporated into their portfolio of accredited methods.

A flexible scope of accreditation therefore enables testing laboratories to react quickly to customer demand and to cope with the large number of new methods, which have to be introduced in the laboratory. Precisely defined procedures for the validation of methods, together with performance and acceptance criteria, are the key points for flexible scope of accreditation.

Finally, Measurement Uncertainty (MU) is an important element in the assessment of validated methods. MU is commonly applied to quantitative measurements (= the estimation of a target concentration), but the concept will also apply to qualitative methods (i.e. confirmation of presence/absence of a target). MU, which should take account of all effects on a measurement process, is the most important single parameter that describes

the quality of measurements. Accreditation standard EN ISO/IEC 17025 requires that a testing laboratory shall have a procedure to estimate MU; the estimation of MU is considerably facilitated, and more rigorous, when well-recognised (validated) methods specify the values of the major sources of uncertainty of measurement; in the case of methods validated in collaborative trials, the value of Reproducibility Standard Deviation (RSD_R), reflecting the inter-laboratory variability, is a useful indicator of the upper overall uncertainty expected for that particular measurement. Validated methods, reporting an estimation of the method variability (i.e. precision) are therefore a useful tool for the establishment of MU in each laboratory.

The JRC IRMM (Institute for Reference Materials and Measurements) published in 2009 a "Guidance Document on Measurement Uncertainty for GMO testing laboratories" (EUR 22756 EN). This JRC report outlines the technical issues related to the estimation of measurement uncertainty involved in the GMO sector. In particular it gives guidance to GMO testing laboratories how to estimate the analytical variability of quantitative analytical results obtained by real-time PCR.

4. Selection of method included in the Compendium of reference methods

This "Compendium of Reference Methods for GMO Analysis" aims at providing a technical state of the art of the detection methods applied in GMO analysis that have been validated according to international standards.

Article 32(1) of Regulation (EC) No 882/2004 (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 laboratories for feed and food are responsible, amongst others, for "providing national reference laboratories with details of analytical methods, including reference methods". However the concept of "reference methods" per se is not strictly defined in EU legislation on GMOs.

The approach taken to select methods eligible for inclusion in this "Compendium of Reference Methods for GMO Analysis" has therefore been the following:

To date a broad range of validated methods are applied in GMO detection, including qualitative and quantitative methods and both protein- or DNA-based technologies. Considering this large diversity of technologies and taking into account the largest common denominator in the global framework of methodology applied in GMO analysis, a first decision taken was to focus this first issue of the "Compendium on Reference Methods for GMO Analysis" (2010 edition) on Polymerase Chain Reaction (PCR) methods i.e. DNA-based detection methods. In follow-up editions, it is foreseen to extend the scope of this "Compendium of Reference Methods for GMO Analysis" to include also DNA-extraction methods and protein-based detection methods.

The methods collected in the current Compendium were also selected based on their reported compliance with ISO 5725 international standard [Accuracy (trueness and precision) of measurement methods and result] and/or the IUPAC (International Union of Pure and Applied Chemistry) "Protocol for the design, conduct and interpretation of method performance studies" (Horwitz, W., *Pure & Appl. Chem.*, 67, 331-343, 1995).

In short, all methods included in the current Compendium (2010 edition) are therefore:

- DNA-based detection methods
- Validated through a collaborative trial according to the principles of ISO 5725 and/or the IUPAC protocol

Typically collaborative trials conducted according to the principles of ISO 5725 or of the IUPAC protocol include a number of laboratories different from the entity that developed and optimised the method under validation. For instance this Compendium includes all methods that were validated by the EURL-GMFF, in collaboration with the ENGL, in the frame of the EU legal provisions on placing on the market of GMOs (see before).

Validation studies require considerable effort and are therefore conducted only on methods that received adequate prior testing. Various organisations and international bodies have addressed the different aspects and requirements for the design and conduction of validation studies. Generally speaking, several aspects have to be taken into account, from the selection of participating laboratories, to the preparation of samples, experimental design, data analysis and reporting. It is of paramount importance that validation studies are designed and conducted in agreement with international standards, given the complexity of the factors involved.

For qualitative methods, for which the full application of the above cited standards is not possible, the general principles and definitions were considered as minimum requirement for inclusion in this Compendium; these include the availability of critical parameters such as specificity, and the compliance with provisions on e.g. number of participating laboratories and selection and preparation of materials used in the experiment.

Additionally, the indications provided in the Codex Alimentarius "Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods" (ALINORM 10/33/23 Appendix III) were taken into account, specifically with regards to Annex III (Validation of a Qualitative PCR Method). These Codex guidelines were approved by the 33rd Session of the Codex Alimentarius Commission in July 2010.

For the methods for which the documentation available to the EURL-GMFF, including validation reports and literature, did not clearly mention that the validation study was performed according to the standards and protocols mentioned above, the coordinator of the validation was contacted and asked to provide information on the protocols followed and the conduction of the study; only upon confirmation that the study was performed in line with the guidelines, the method was included in this Compendium.

Once selected according to the above criteria, a list of 79 reference methods has been identified.

Each method has then been described in a comprehensive summary which provides the essential information related to the validated method. Not all details are given for each method but all necessary references are provided for further information about each method.

Comments on the present edition are welcome ahead of publication of updated and revised versions of the "Compendium of Reference Methods for GMO Analysis".

5. Method Compendium structure and Method Data Sheet content: a summary explanation

This Compendium groups GMO detection methods validated by collaborative trials organised by research organisations from all over the world during the period 1999 to 2009.

It contains basically two distinct types of PCR methods: a set of quantitative methods and a set of qualitative methods.

Both sets have been grouped in a distinct chapter. In total 49 quantitative methods and 30 qualitative PCR methods are documented in this Compendium.

In both cases, these detection methods may target various types of DNA sequences in the GMO: so-called 'trait/gene/promoter-specific' sequences (ELE), 'construct-specific' sequences (CON), 'event-specific' sequences (EV) and 'taxon- or species-specific' sequences (TAX).

'Trait/gene/promoter-specific' sequences (ELE) are DNA sequences that are solely confined to one particular molecular entity (such as the CaMV 35S promoter, the coding region of the CryIAb gene ...).

The 'construct-specific sequences (CON) are DNA sequences that span two different types of molecular entities, such as a promoter sequence and a trait sequence.

The 'event-specific sequences' (EV) are a special type of 'construct-specific' elements allowing to univoquely identify the presence of one particular GMO. This kind of DNA sequences typically contain part of the host genome linked to the inserted recombinant sequence.

Finally, 'taxon- or species-specific' (TAX) sequences represent DNA sequences that are confined to a particular taxon or species. These sequences allow identifying the composition of the material and are often used as the divisor in the quantification of the GMO content of a product.

The chapter on the quantitative methods covers all event-specific methods validated by the EURL-GMFF within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed and a number of PCR methods validated by other research institutes. As indicated above, only methods meeting the IUPAC or the ISO 5725 criteria have been retained in this document.

Each quantitative PCR method has received a unique Compendium reference number 'QT/XX/000', wherein:

- QT stands for 'quantitative'
- the 'XX' provides a capitalized binomial species abbreviation in case of an event-specific method or an annotation referring to the type of target ('CON' for 'construct-specific' sequences, 'ELE' for 'trait/gene/promoter-specific' sequences, or 'TAX' for 'taxon- or species-specific' sequences)
- the digit number of each method was attributed according to its date of publication.

The qualitative methods retained in the Compendium are essentially applicable in screening for the presence of a target in the sample, and as such referred to in the Compendium reference number 'SC/XXX/000' wherein:

- the 'SC' refers to their use in screening approaches
- the 'XXX' equals 'CON' for the 'construct-specific' sequences, 'ELE' for 'trait/gene/promoter-specific' sequences, 'EV' for 'event-specific' sequences and 'TAX' for 'taxon- or species-specific' sequences
- the digit number of each method was attributed according to its date of publication.

For each method, the descriptive sheet is structured in a similar way:

1. General Information
2. Validation Data
3. References
4. Primers and Probes Sequences
5. PCR reaction setup
6. Amplification conditions

For the quantitative methods, the following parameters were considered as key parameters: the Limit of Detection (LOD) and the Limit of Quantification (LOQ), the test levels, the mean values obtained,

the mean slope and PCR efficiency of the respective PCR methods, and the precision (RSD_r, RSD_R, R^2). For the qualitative methods, in addition to the above parameters, reporting false positive and false negative ratios were included. A definition on all these terms has been included in the glossary table below.

For any further information on these methods, we refer to the following websites:

http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm

http://mbg.jrc.ec.europa.eu/home/ict/methods-database.htm

Glossary on the definitions of the Method Performance Parameters

The purpose of a collaborative trial is to verify the transferability and performance of a method among laboratories, according to the principles of either the IUPAC harmonised Protocol[1] or ISO5725[2] or Codex Alimentarius [3].

Below the definition of the method performance parameters applied in this Compendium as formulated in the above mentioned documents or by he European Network of GMO Laboratories (ENGL)[4].

Method performance Parameters:

False positive:
The probability that a known negative sample is classified as positive, for convenience this rate is expressed as a percentage.

> % False positive results = number of misclassified known negative samples / total number of known negative samples

False negative
The probability that a known positive sample is classified as negative, for convenience this rate is expressed as a percentage.

> % False negative results = number of misclassified known positive samples / total number of known positive samples

Sensitivity
The sensitivity of a method is a measure of the magnitude of the response caused by a certain amount of analyte.

Specificity
Property of a method to respond exclusively to the characteristic or analyte of interest.

Bias/Trueness
The closeness of agreement between the average values obtained from a large series of test results and an accepted reference value. The measure of trueness is usually expressed in terms of bias.

Slope and PCR Efficiency
The rate of amplification that leads to a theoretical slope of –3.32 with an efficiency of 100% in each cycle. The efficiency of the reaction can be calculated by the following equation:

$$Efficiency = 10^{\left(\frac{-1}{slope}\right)} - 1$$

R^2 Coefficient
The R^2 coefficient is the correlation coefficient of a standard curve obtained by linear regression analysis.

Precision - Relative Repeatability Standard Deviation (RSDr)
The relative standard deviation of test results obtained under repeatability conditions. Repeatability conditions are conditions where test results are obtained with the same method, on identical test items, in the same laboratory, by the same operator, using the same equipment within short intervals of time.

Precision - Relative Reproducibility Standard Deviation (RSD_R)
The relative standard deviation of test results obtained under reproducibility conditions. Reproducibility conditions are conditions where test results are obtained with the same method, on identical test items, in different laboratories, with different operators, using different equipment. Reproducibility standard deviation describes the inter-laboratory variation.

Limit of Quantification (LOQ)
The limit of quantification is the lowest amount or concentration of analyte in a sample that can be reliably quantified with an acceptable level of precision and accuracy.

Limit of Detection (LOD)
The limit of detection is the lowest amount or concentration of analyte in a sample, which can be reliably detected, but not necessarily quantified, as demonstrated by single-laboratory validation.

References
(1) IUPAC Protocol for the Design, Conduct and Interpretation of Method Performance Studies (1995) Pure & Appl. Chem., 67, 331-343
(2) International Standard (ISO) 5725 (1994) Accuracy (trueness and precision) of measurement methods and results. International Organization for Standardisation, Genève, Switzerland
(3) Codex Alimentarius Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (2010), ALINORM 10/33/23 Appendix III
(4) Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing European Network of GMO Laboratories (ENGL) (2009)

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Chapter 1:
Quantitative GMO detection PCR methods

Quantitative PCR method for detection of maize event Bt11

1. GENERAL INFORMATION

Target genetic element	Junction region between the Intron 6 (IVS6) from maize alcohol dehydrogenase 1 gene (adh1-1S) and a synthetic cryIA(b) gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/001

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	maize flour
Materials used for calibration/controls	plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	Bt11
Unique Identifier	SYN-BT011-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.5%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.50%**	**1.0%**	**5.0%**	**10%**
Mean Value (%)	0.09%	0.51%	1.2%	6.1%	12%
RSD_r (%)	22%	24%	19%	14%	10%
RSD_R (%)	18%	21%	19%	13%	12%
Bias %	-9.0%	2.0%	15%	22%	21%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The absolute LOD and LOQ values were not determined in this collaborative trial.

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AAAAGACCACAACAAGCCGC-3'
Target element	*IVS 6*
Primer Reverse	5'-CAATGCGTTCTCCACCAAGTACT-3'
Target element	cryIA(b)
Amplicon length	127 bp
Probe	5'-FAM-CGACCATGGACAACAACCCAAACATCA-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CTCCCAATCCTTTGACATCTGC-3'
Target element	*zSSIIb*
Primer Reverse	5'-TCGATTTCTCTCTTGGTGACAGG-3'
Target element	*zSSIIb*
Amplicon length	151 bp
Probe	5'-FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA-3'
Target element	maize starch synthase Iib (*zSSIIb*) gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMul5

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing & Extension	59°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event Bt11

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event Bt11 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/006

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM event Bt11 sweet maize
Tested GM events	
Event Name	Bt11
Unique Identifier	SYN-BT011-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received twelve blind samples (six pairs of blind duplicate DNA samples) representing six GM levels, namely 0.1%, 0.3%, 0.7% 1.0%, 1.3% and 2% of Bt11 sweet maize DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, negative target controls consisting of non-GM maize DNA and Bt176 maize DNA extracted from Certified Reference Material, primers and probes for the alcohol dehydrogenase (*adh1)* reference gene and for the Bt11 specific system. Two replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.1%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.1%	0.3%	0.7%	1%	1.3%	2.0%
Mean Value (%)	0.1%	0.3%	0.7%	1%	1.2%	1.8%
RSD_r (%)	34%	19%	24%	10%	25%	15%
RSD_R (%)	34%	19%	24%	13%	27%	18%
Bias %	n.a	n.a	n.a	n.a	n.a	n.a

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The relative LOD and LOQ values were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Puumalaainen J, Van Den Eede G. Validation of the GMO Specific Detection Method Developed by NVI/INRA for Bt11 in Sweet Corn Maize - Validation Report and Protocol. EUR 21829 EN. 2005. JRC32111 (ISBN 92-79-00110-8)

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCGGAACCCCTATTTGTTTA-3'
Target element	Insert
Primer Reverse	5'-TCCAAGAATCCCTCCATGAG-3'
Target element	3' junction
Amplicon length	70 bp
Probe	5'-FAM-AAATACATTCAAATATGTATCCGCTCA-TAMRA-3'
Probe Name	Bt113JFT
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-FAM-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Probe Name	ADH1-MDO
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
10X TaqMan® Buffer A	1x	TaqMan® Universal PCR Master Mix	1x
$MgCl_2$	4 mmol/L	Primer Fw	0,30 µmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	Primer Rev	0,30 µmol/L
dUTP	400 µmol/L	Probe	0,20 µmol/L
Primer Fw	0,75 µmol/L	Nuclease-free water	#
Primer Rev	0,75 µmol/L	Template DNA	maximum 250
Probe	0,25 µmol/L	Final Volume	25 µL
AmpErase® UNG	0,3 U		
AmpliTaq Gold® DNA Polymerase	1,5 U		
Nuclease-free water	#		
Template DNA	maximum 200		
Final Volume	25 µL		

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			50

Quantitative PCR method for detection of maize event Bt11

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event Bt11 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/015

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event Bt11 seeds
Tested GM events	
Event Name	Bt11
Unique Identifier	SYN-BT011-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 5% and 8% of maize event Bt11 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenas 1 (*adh1)* reference gene and for the Bt11 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.40%	0.90%	5.0%	8.0%
Mean Value (%)	0.09%	0.39%	0.92%	4.7%	7.9%
RSD_r (%)	17%	13%	11%	13%	9%
RSD_R (%)	24%	16%	15%	15%	14%
Bias %	2.2%	-1.9%	1.8%	-5.2%	-1.2%

	GMO Target
Mean Slope	-3.5
Mean PCR Efficiency %	93
Mean R^2	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Larcher S, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Event Bt11 Using Real-time PCR. Validation Report and Protocol EUR 23649 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48909 (ISBN 978-92-79-11049-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGTGTGGCCATTTATCATCGA-3'
Target element	5'-host genome
Primer Reverse	5'-CGCTCAGTGGAACGAAAACTC-3'
Target element	Insert
Amplicon length	68 bp
Probe	5'-FAM-TTCCATGACCAAAATCCCTTAACGTGAGT-TAMRA-3'
Probe Name	Bt11-ev-p1
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-VIC-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Probe Name	Zm adh1-P
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
JumpStart™ Taq ReadyMix™ (Sigma)	1x	JumpStart™ Taq ReadyMix™ (Sigma)	1x
Primer Fw	0,20 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,15 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 250	Template DNA	maximum 250
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event Bt 176

1. GENERAL INFORMATION

Target genetic element	Junction region between a synthetic *cryIA(b)* gene and the Phosphoenol-pyruvate Carboxylase (PEPC) intron N.9
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/002

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	maize flour
Materials used for calibration/controls	plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	Bt 176
Unique Identifier	SYN-EV176-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.1%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.50%**	**1.00%**	**5%**	**10.0%**
Mean Value (%)	0.11%	0.49%	0.92%	5%	9.6%
RSD_r (%)	16%	5.8%	7.1%	8.1%	5.8%
RSD_R (%)	21%	10%	11%	11%	9.5%
Bias %	11%	-1.6%	-7.7%	0%	-3.8%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The relative LOD and LOQ values validated in the collaborative trial corresponded to 0.1% GMO (w/w).

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGTTCACCAGCAGCAACCAG-3'
Target element	*cryIA(b)*
Primer Reverse	5'-ACTCCACTTTGTGCAGAACAGATCT-3'
Target element	*IVS 9 PEPC*
Amplicon length	100 bp
Probe	5'-FAM-CCGACGTGACCGACTACCACATCGA-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CTCCCAATCCTTTGACATCTGC-3'
Target element	*zSSIIb*
Primer Reverse	5'-TCGATTTCTCTCTTGGTGACAGG-3'
Target element	*zSSIIb*
Amplicon length	151 bp
Probe	5'-FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA-3'
Target element	maize starch synthase IIb (*zSSIIb*) gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMul5

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing & Extension	59°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event 59122

1. GENERAL INFORMATION

Target genetic element	Integration border region between the insert of maize event 59122 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/012

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted non-GM and GM maize event 59122 seeds
Tested GM events	
Event Name	59122
Unique Identifier	DAS-59122-7
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing 5 GM levels, namely 0.1%, 0.4%, 0.9%, 2.0% and 4.5% of maize event DAS-59122-7 DNA in non-GM maize DNA. In addition the laboratories received four calibration samples, an amplification reagent control, reaction reagents, primers and probes for the high-mobility-group A (*hmgA*) reference gene and the DAS-59122-7 maize specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	0.045%	**LOD Absolute**	not reported
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.0%	4.5%
Mean Value (%)	0.13%	0.46%	0.98%	2.1%	4.4%
RSD_r (%)	18%	14%	16%	14%	8.5%
RSD_R (%)	25%	22%	22%	15%	13%
Bias %	29%	15%	9%	7%	-1%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	91	93
Mean R^2	0.99	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Grazioli E, Larcher S, Savini C, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line DAS-59122-7 Using Real-Time PCR - Validation Report and Protocol. EUR 22133 EN. 2006. JRC32187 (ISBN 92-79-01535-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GGGATAAGCAAGTAAAAGCGCTC-3'
Target element	not specified
Primer Reverse	5'-CCTTAATTCTCCGCTCATGATCAG-3'
Target element	not specified
Amplicon length	86 bp
Probe	5'-FAM-TTTAAACTGAAGGCGGGAAACGACAA-TAMRA-3'
Probe Name	DAS-59122-7-rb1rs-
Target element	DNA sequence in the IBR

Taxon-target(s)

Primer Forward	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Primer Reverse	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Probe Name	Mhmg-probe
Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer II (10x)	1x	PCR buffer II (10x)	1x
ROX™ reference dye	0,7x	ROX™ reference dye	0,7x
Tween-20	0,01%	Tween-20	0,01%
Glycerol	0,8%	Glycerol	0,8%
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
$MgCl_2$	5,0 mmol/L	$MgCl_2$	5,5 mmol/L
Primer Fw	0,25 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,25 µmol/L	Primer Rev	0,40 µmol/L
Probe	0,20 µmol/L	Probe	0,15 µmol/L
AmpliTaq Gold® DNA Polymerase	0,04 U/µL	AmpliTaq Gold® DNA Polymerase	0,04 U/µL
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			50

Quantitative PCR method for detection of maize event GA21

1. GENERAL INFORMATION

Target genetic element	Junction region between an optimized transit peptide sequence (OTP) and point mutated *epsps* gene (mEPSPS) from maize
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/003

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	GA21
Unique Identifier	MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.1%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	**0.1%**	**0.50%**	**1.0%**	**5.0%**	**10%**
Mean Value (%)	0.1%	0.54%	1.2%	5.8%	12%
RSD_r (%)	21%	13%	12%	8.2%	7.9%
RSD_R (%)	21%	22%	19%	16%	14%
Bias %	-5.4%	7.7%	20%	17%	15%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The absolute LOD and LOQ values were not determined in the collaborative trial.

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GAAGCCTCGGCAACGTCA-3'
Target element	*OTP*
Primer Reverse	5'-ATCCGGTTGGAAAGCGACTT-3'
Target element	*mEPSPS*
Amplicon length	133 bp
Probe	5'-FAM-AAGGATCCGGTGCATGGCCG-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CTCCCAATCCTTTGACATCTGC-3'
Target element	*zSSIIb*
Primer Reverse	5'-TCGATTTCTCTCTTGGTGACAGG-3'
Target element	*zSSIIb*
Amplicon length	151 bp
Probe	5'-FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA-3'
Target element	maize starch synthase IIb (*zSSIIb*) gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMul5

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing & Extension	59°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event GA21

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event GA21 and the host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/007

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	Certified Reference Material IRMM-414 (JRC-IRMM)
Tested GM events	
Event Name	GA21
Unique Identifier	MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 12 blind maize flour samples representing 6 GM levels, namely 0.1%, 0.49%, 0.98%, 1.3%, 1.71% and 4.26% of maize event GA21 in non-GM maize (w/w). These samples were prepared by the IRC-IRMM. In addition, the laboratories received a calibration maize flour sample containing 4.26% of GA21, two negative DNA target controls consisting of Bt 176 maize DNA and in non-GM maize flour, reaction reagents, primers and probes for the alcohol dehydrogenas 1 (*adh1)* reference gene and for the GA21 specific system. For each unknown sample and for the calibration sample, the laboratories performed an enhanced CTAB DNA extraction, a spectrophotometric quantification of the amount of DNA extracted, a real-time PCR monitor run (inhibition test) and a quantitative real-time PCR analysis. Samples were analyzed in parallel with both the reference and the transgenic specific system. The standard and control samples were analyzed in triplicates, the unknown samples in quadruplicates. The two replicates for each GM level were analyzed in two separate runs.

Method Performance

LOD Relative	≤ 0.05%	**LOD Absolute**	not reported
LOQ Relative	0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.49%	0.98%	1.3%	1.7%	4.3%
Mean Value (%)	0.16%	0.67%	1.20%	1.6%	2.1%	4.6%
RSD_r (%)	24%	26%	20%	19%	21%	16%
RSD_R (%)	44%	35%	29%	31%	27%	30%
Bias %	55.0%	36%	18%	26%	21%	8.9%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Paoletti C, Mazzara M, Puumalaainen J, Rasulo D, Van Den Eede G. Validation of an Event-Specific Method for the Quantitation of Maize Line GA21 Using Real-Time PCR. EUR 21520 EN. 2005. JRC32087 (ISBN 92-894-9184-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3'
Target element	5'-host genome
Primer Reverse	5'-TGGCTCGCGATCCTCCT-3'
Target element	Insert
Amplicon length	112 bp
Probe	5'-FAM-CATATACTAACTCATATCTCTTTCTCAACAGCAGGTGGGT-TAMRA-3'
Probe Name	GA21 probe PR
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCCTCATGGCCAAAG-3'
Target element	*adh1*
Primer Reverse	5'-CCTTCTTGGCGGCTTATCTG-3'
Target element	*adh1*
Amplicon length	70 bp
Probe	5'-FAM-CTTAGGGGCAGACTCCCGTGTTCCCT-TAMRA-3'
Probe Name	*adh1* probe PR
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	200 ng	Template DNA	200 ng
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event GA21

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event GA21 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/014

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event GA21
Tested GM events	
Event Name	GA21
Unique Identifier	MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received twenty blind samples, representing five GM levels, namely 0.09%, 0.5%, 0.9%, 5% and 8% of GA21 maize DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenase (*adh1)* reference gene and for the GA21 specific system. For replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤0.04%	**LOD Absolute**	not reported
LOQ Relative	≤0.04%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.5%	0.90%	5.0%	8.0%
Mean Value (%)	0.08%	0.5%	0.91%	4.7%	7.3%
RSD_r (%)	23%	17%	20%	20%	17%
RSD_R (%)	23%	21%	21%	24%	20%
Bias %	-8.7%	0.8%	1.6%	-5.6%	-8.5%

	GMO Target
Mean Slope	-3.4
Mean PCR Efficiency %	98
Mean R^2	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Larcher S, Savini C, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Line GA21 Using Real-time PCR - Validation Report and Protocol - Report on the Verification of Performance of a DNA Extraction Method for Maize Grains. EUR 23090 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC42964 (ISBN 978-92-79-08193-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CGTTATGCTATTTGCAACTTTAGAACA-3'
Target element	5'-host genome
Primer Reverse	5'-GCGATCCTCCTCGCGTT-3'
Target element	Insert
Amplicon length	101 bp
Probe	5'-FAM-TTTCTCAACAGCAGGTGGGTCCGGGT-TAMRA-3'
Probe Name	esGA21-5' probe
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-VIC-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Probe Name	Zm Adh1 probe
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
JumpStart™ Taq ReadyMix™ (Sigma)	1x	JumpStart™ Taq ReadyMix™ (Sigma)	1x
Primer Fw	0,90 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,90 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 250	Template DNA	maximum 250
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event LY038

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event LY038 and the host maize genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/017

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event LY038 seeds
Tested GM events	
Event Name	LY038
Unique Identifier	REN-00038-3
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.09%, 0.5%, 0.9%, 5.0% and 8.0% of maize event LY038 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the high-mobility-group A (*hmgA*) reference gene and for the LY038 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.5%	0.90%	5.0%	8.0%
Mean Value (%)	0.09%	0.5%	0.88%	4.7%	7.9%
RSD_r (%)	25%	16%	9.3%	18%	12%
RSD_R (%)	35%	21%	20%	23%	26%
Bias %	-2.7%	-0.4%	-2.0%	-6.7%	-0.7%

	GMO Target	Taxon Target
Mean Slope	-3.6	-3.2
Mean PCR Efficiency %	90	108
Mean R^2	0.99	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Grazioli E, Larcher S, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Line LY038 Using Real-time PCR - Validation Report and Protocol. EUR 23647 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48919 (ISBN 978-92-79-11047-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGGGTTCAGTCTGCGAATGTT-3'
Target element	5'-host genome
Primer Reverse	5'-AGGAATTCGATATCAAGCTTATCGA-3'
Target element	Insert
Amplicon length	111 bp
Probe	5'-FAM-CGAGCGGAGTTTATGGGTCGACGG-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Target element	high-mobility-group A *(hmgA)* gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	10X TaqMan® Buffer A	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,05 µmol/L	Probe	0,16 µmol/L
Nuclease-free water	#	$MgCl_2$	6,5 mmol/L
Template DNA	maximum 200	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L
Final Volume	50 µL	Nuclease-free water	#
		Template DNA	maximum 200
		Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event MIR604

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MIR 604 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/013

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples from non-GM and GM maize event MIR604
Tested GM events	
Event Name	MIR604
Unique Identifier	SYN-IR604-5
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.5% and 6.0% of maize event MIR604 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the alcohol dehydrogenase 1 (*adh1)* reference gene and for the MIR604 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	< 0.045%	**LOD Absolute**	not reported
LOQ Relative	< 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.1%	0.40%	0.90%	2.5%	6.0%
Mean Value (%)	0.1%	0.41%	0.89%	2.5%	5.8%
RSD_r (%)	24%	17%	12%	16%	14%
RSD_R (%)	27%	18%	18%	22%	20%
Bias %	3.6%	3.1%	-1.0%	0.7%	-3.6%

	GMO Target
Mean Slope	-3.3
Mean PCR Efficiency %	97
Mean R^2	1.00

Comment
The LOD and LOQ values were provided by the applicant and were not assessed in the collaborative trail.

3. REFERENCES

Mazzara M, Munaro B, Foti N, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Maize Line MIR604 Using Real-time PCR - Validation Report and Protocol - Maize Seeds Sampling and DNA Extraction. EUR 22913 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37490 (ISBN 978-92-79-06930-7)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCGCACGCAATTCAACAG-3'
Target element	5'-host genome
Primer Reverse	5'-GGTCATAACGTGACTCCCTTAATTCT-3'
Target element	Insert
Amplicon length	76 bp
Probe	5'-FAM-AGGCGGGAAACGACAATCTGATCATG-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-VIC-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
JumpStart™ Taq ReadyMix™ (Sigma)	1x	JumpStart™ Taq ReadyMix™ (Sigma)	1x
Primer Fw	0,60 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	Maximum 200	Template DNA	maximum 250
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event MON 810

1. GENERAL INFORMATION

Target genetic element	Junction region between the Intron 1 from the maize *hsp70* gene (IVS-HSP) and a synthetic *cryIA(b)* gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/004

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	maize flour
Materials used for calibration/controls	plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	MON810
Unique Identifier	MON-00810-6
Crop Name	*Zea mays* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.5%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.50%**	**1.0%**	**5.0%**	**10.0%**
Mean Value (%)	0.13%	0.55%	1.1%	4.8%	9.8%
RSD_r (%)	32%	15%	12%	14%	11%
RSD_R (%)	26%	20%	15%	12%	12%
Bias %	25%	9.4%	4.6%	-4.3%	-1.8%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The relative LOD and LOQ values validated in the collaborative trial corresponded respectively to 0.1% and 0.5% GMO (w/w).

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GATGCCTTCTCCCTAGTGTTGA-3'
Target element	*IVS-HSP*
Primer Reverse	5'-GGATGCACTCGTTGATGTTTG-3'
Target element	*cryIA(b)*
Amplicon length	113 bp
Probe	5'-FAM-AGATACCAAGCGGCCATGGACAACAA-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CTCCCAATCCTTTGACATCTGC-3'
Target element	*zSSIIb*
Primer Reverse	5'-TCGATTTCTCTCTTGGTGACAGG-3'
Target element	*zSSIIb*
Amplicon length	151 bp
Probe	5'-FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA-3'
Target element	maize starch synthase IIb (*zSSIIb*) gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMul5

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing & Extension	59°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event MON 810

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MON 810 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/020

2. VALIDATION DATA

Collaborative trial coordinator	Federal Institute for Risk assessment (BfR)
Test material applied in collaborative trial	Certified Reference Material (maize flour)
Materials used for calibration/controls	CRM IRMM-413 (JRC-IRMM)
Tested GM events	
Event Name	MON 810
Unique Identifier	MON-00810-6
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participant received 12 blind duplicate samples consisting of certified reference material (CRM IRMM-413) representing six GM levels, namely <0.02%, 0.1%, 0,5%, 1%, 2%, and 5% of GM MON 810 (mass fraction) in conventional maize. Each sample was extracted once and analysed by PCR in tree replicates. For relative quantification of MON 810 maize, the high-mobility-group A (*hmgA*) gene was targeted with specific primers.

Method Performance

LOD Relative	≤ 0.1%	**LOD Absolute**	5 HGE
LOQ Relative	≤ 0.1%	**LOQ Absolute**	10 HGE

Values determined in the collaborative trial

Test Level (%)	**< 0.02%**	**0.1%**	**0.50%**	**1.00%**	**2.0%**	**5.0%**
Mean Value (%)	0.03%	0.1%	0.46%	0.83%	1.8%	4.5%
RSD_r (%)	26%	36%	21%	17%	16%	29%
RSD_R (%)	83%	45%	44%	32%	32%	37%
Bias %	>40%	2.3%	-7.7%	-17%	-11%	-9.7%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The absolute and relative LOD and LOQ values were not reported in the collaborative trial. This trial was performed in collaboration with The American Association of Cereal Chemists (AACC), the JRC-IRMM, the JRC-IHCP and GeneScan.

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Report on the Verification of the Performance of a MON810 Event-specific Method on Maize Line MON810 Using Real-time PCR. EUR 24237 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56609 (ISBN 978-92-79-14982-5)

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TCGAAGGACGAAGGACTCTAACGT-3'
Target element	5'-host genome
Primer Reverse	5'-GCCACCTTCCTTTTCCACTATCTT-3'
Target element	Insert
Amplicon length	92 bp
Probe	5'-FAM-AACATCCTTTGCCATTGCCCAGC-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
AmpliTaq Gold® DNA Polymerase	1,25 U	AmpliTaq Gold® DNA Polymerase	1,25 U
AmpErase® UNG	0,5 U	AmpErase® UNG	0,5 U
TaqMan buffer A (with ROX™)	1x	TaqMan buffer A (with ROX™)	1x
$MgCl_2$	6,5 mmol/L	$MgCl_2$	6,5 mmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
Primer Fw	0,30 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,18 µmol/L	Probe	0,16 µmol/L
Template DNA	2,3-150 ng	Template DNA	2,3-150 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event MON 863

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MON 863 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/009

2. VALIDATION DATA

Collaborative trial Coordinator	JRC-IHCP
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	CRM IRMM-413 (JRC-IRMM)
Tested GM events	
Event Name	MON 863
Unique Identifier	MON-00863-5
Species	*Zea mays* L.

Collaborative trial Description

The participants received 10 blind maize flour samples representing 5 GM levels, namely 0.%, 0.1%, 1.0%, 5.0% and 10.0% of maize event MON 863 in non-GM maize (w/w), prepared by the JRC-IRMM, a sample for calibration, (10% MON 863 maize flour), two negative DNA target controls (Bt176 maize DNA and non-GM maize flour) and reagents. For each blind and calibration sample, a CTAB DNA extraction, followed by spectrophotometric quantification, a real-time PCR monitor run (inhibition test) and a quantitative real-time PCR analysis was performed. Samples were analysed in triplicate (calibrators) or in quadruplicate (blind) on the same plate with both the reference and the transgenic specific system. Two replicates for each GM level were analysed in two separate runs.

Method Performance

Values determined in the collaborative trial

Level	**0.10%**	**1.0%**	**5%**	**10.0%**	**0%**
Mean Value	0.13%	1.2%	5%	9.4%	0%
RSD_r (%)	35%	17%	10%	13%	-
RSD_R (%)	35%	18%	18%	21%	-
Bias %	28%	20%	0%	-6%	-

	GMO Target	Taxon Target
Mean Slope	-3.87	-3.62
Mean PCR Efficiency %	84	88
Mean Linearity (R2)	0.97	0.97

3. REFERENCES

Mazzara M, Foti N, Price S, Paoletti C, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line MON 863 Using Real-Time PCR - Validation Report and Protocol. EUR 21830 EN. 2005. JRC32105 (ISBN 92-79-00111-6)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTAGGATCGGAAAGCTTGGTAC-3'
Target element	5'-host genome
Primer Reverse	5'-TGTTACGGCCTAAATGCTGAACT-3'
Target element	Insert
Amplicon	84 bp
Probe	5'-FAM-TGAACACCCATCCGAACAAGTAGGGTCA-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCCTCATGGCCAAAG-3'
Target element	*adh1*
Primer Reverse	5'-CCTTCTTGGCGGCTTATCTG-3'
Target element	*adh1*
Amplicon	70 bp
Probe	5'-FAM-CTTAGGGGCAGACTCCCGTGTTCCCT-TAMRA-3'
Target element	alcohol dehydrogenase 1 (*adh1)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,050 µmol/L	Probe	0,050 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 280	Template DNA	maximum 280
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event MON 88017

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event MON 88017 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/016

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event MON 88017 seeds
Tested GM events	
Event Name	MON 88017
Unique Identifier	MON-88017-3
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.09%, 0.5%, 0.9%, 5.0% and 8.0% of maize event MON 88017 DNA in non-GM maize DNA. In addition, the laboratories received five calibration samples, amplification reagent control, reaction reagents, primers and probes for the high-mobility-group A (*hmgA*) reference gene and for the MON 88017 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.50%	0.90%	5.0%	8.0%
Mean Value (%)	0.09%	0.51%	0.81%	4.8%	7.4%
RSD_r (%)	28%	13%	19%	19%	18%
RSD_R (%)	33%	28%	23%	27%	23%
Bias %	-2.6%	2.9%	-9.6%	-4.8%	-7.6%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.2
Mean PCR Efficiency %	94	107
Mean R^2	0.99	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Foti N, Grazioli E, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Line MON 88017 Using Real-time PCR - Validation Report and Protocol. EUR 23646 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48920 (ISBN 978-92-79-11046-7)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GAGCAGGACCTGCAGAAGCT-3'
Target element	Insert
Primer Reverse	5'-TCCGGAGTTGACCATCCA-3'
Target element	3'-host genome
Amplicon length	95 bp
Probe	5'-FAM-TCCCGCCTTCAGTTTAAACAGAGTCGGGT-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	10X TaqMan® Buffer A	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,05 µmol/L	Probe	0,16 µmol/L
Nuclease-free water	#	$MgCl_2$	6,5 mmol/L
Template DNA	maximum 200	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L
Final Volume	50 µL	Nuclease-free water	#
		Template DNA	maximum 200
		Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event MON 89034

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event MON 89034 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/018

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event MON 89034 seeds
Tested GM events	
Event Name	MON 89034
Unique Identifier	MON-89034-3
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 3.0% and 8.0%% of maize event MON 89034 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the high-mobility-group A (*hmgA*) reference gene and for the MON 89034 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.40%	0.90%	3.0%	8.0%
Mean Value (%)	0.11%	0.43%	0.94%	2.8%	7.2%
RSD_r (%)	18%	13%	17%	12%	9.5%
RSD_R (%)	22%	15%	17%	14%	10%
Bias %	25%	6.4%	4.3%	-5.8%	-11%

	GMO Target	Taxon Target
Mean Slope	-3.6	-3.3
Mean PCR Efficiency %	89	101
Mean R^2	0.99	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Savini C, Bogni A, Grazioli E, Munaro B, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Line MON 89034 Using Real-time PCR - Validation Report and Protocol. EUR 23700 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48921 (ISBN 978-92-79-11166-2)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TTCTCCATATTGACCATCATACTCATT-3'
Target element	Insert
Primer Reverse	5'-CGGTATCTATAATACCGTGGTTTTTAAA-3'
Target element	3'-host genome
Amplicon length	77 bp
Probe	5'-FAM-ATCCCCGGAAATTATGTT-MGBNFQ-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmg*A
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmg*A
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® PCR Master Mix	1x	10X TaqMan® Buffer A	1x
Primer Fw	0,45 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,45 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,10 µmol/L	Probe	0,16 µmol/L
Nuclease-free water	#	$MgCl_2$	6,5 mmol/L
Template DNA	maximum 200	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each
Final Volume	50 µL	Nuclease-free water	#
		AmpliTaq Gold® DNA Polymerase	1,25 U
		Template DNA	maximum 200
		Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event NK 603

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event NK 603 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/008

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	Certified Reference Material IRMM-415 (JRC-IRMM)
Tested GM events	
Event Name	NK 603
Unique Identifier	MON-00603-6
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 10 blind samples representing 5 GM levels, namely 0.1%, 0.49%, 0.98%, 1.96%, and 4.91% of maize event NK 603 in non-GM maize (w/w). In addition the laboratories received a calibration maize flour sample containing 4.91% of NK 603 maize in non-GM maize (IRMM-415), two negative DNA target controls consisting of maize event Bt 176 DNA and non-GM maize flour, reaction reagents, primers and probes for the *adh1* reference gene and the NK 603 specific system. For each unknown sample and for the calibration sample the laboratories performed an enhanced CTAB DNA extraction, a spectrophotometric quantification of the amount of DNA extracted, a real-time PCR monitor run (inhibition test) and a quantitative real-time PCR analysis. Samples were analyzed in parallel with both the reference and the transgenic specific system. The standard and control samples were analyzed in triplicates, the blind samples in quadruplicates. The two replicates for each GM level were analyzed in two separate runs.

Method Performance

LOD Relative	≤ 0.05%	**LOD Absolute**	not reported
LOQ Relative	0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.49%	0.98%	2.0%	4.9%
Mean Value (%)	0.18%	0.85%	1.4%	2.2%	6.0%
RSD_r (%)	24%	15%	17%	7.7%	22%
RSD_R (%)	37%	34%	25%	26%	31%
Bias %	83.0%	73%	47%	14%	22%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Paoletti C, Puumalaainen J, Rasulo D, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line NK603 Using Real-Time PCR - Validation Report and Protocol. EUR 21825 EN. 2005. JRC32103 (ISBN 92-79-00106-X)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3'
Target element	Insert
Primer Reverse	5'-AAGAGATAACAGGATCCACTCAAACACT-3'
Target element	3'-host genome
Amplicon length	108 bp
Probe	5'-FAM-TGGTACCACGCGACACACTTCCACTC-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCCTCATGGCCAAAG-3'
Target element	*adh1*
Primer Reverse	5'-CCTTCTTGGCGGCTTATCTG-3'
Target element	*adh1*
Amplicon length	70 bp
Probe	5'-FAM-CTTAGGGGCAGACTCCCGTGTTCCCT-TAMRA-3'
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 300	Template DNA	maximum 300
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event T25

1. GENERAL INFORMATION

Target genetic element	Junction region between the phosphinothricin N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes* and CaMV 35S terminator (CaMV T-35S)
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/005

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	T25
Unique Identifier	ACS-ZM003-2
Crop Name	*Zea mays* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.5%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	1.0%	5.0%	10%
Mean Value (%)	0.14%	0.58%	1.2%	5.6%	11%
RSD_r (%)	24%	28%	6.8%	12%	13%
RSD_R (%)	27%	28%	12%	15%	15%
Bias %	39%	15%	20.0%	12%	8.1%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The absolute LOD and LOQ values were not determined in this collaborative trial.

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCCAGTTAGGCCAGTTACCCA-3'
Target element	*pat*
Primer Reverse	5'-TGAGCGAAACCCTATAAGAACCCT-3'
Target element	*CaMV T-35S*
Amplicon length	149 bp
Probe	5'-FAM-TGCAGGCATGCCCGCTGAAATC-TAMRA-3'
Target element	DNA sequence in the junction region

Taxon-target(s)

Primer Forward	5'-CTCCCAATCCTTTGACATCTGC-3'
Target element	*zSSIIb*
Primer Reverse	5'-TCGATTTCTCTCTTGGTGACAGG-3'
Target element	*zSSIIb*
Amplicon length	151 bp
Probe	5'-FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA-3'
Target element	maize starch synthase IIb (*zSSIIb*) gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMul5

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	30"	
Annealing & Extension	59°C	60"	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of maize event T25

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event T25 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/011

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event T25
Tested GM events	
Event Name	T25
Unique Identifier	ACS-ZM003-2
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3 % of maize event T25 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the alcohol dehydrogenase 1 (*adh1*) reference gene and for the T25 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.15%	0.40%	0.90%	2.0%	3.3%
Mean Value (%)	0.11%	0.38%	0.82%	1.8%	3.5%
RSD_r (%)	26%	22%	10%	22%	11%
RSD_R (%)	26%	23%	21%	22%	18%
Bias %	-0.27%	-6.0%	-9.0%	-12%	6%

	GMO Target
Mean Slope	-3.4
Mean PCR Efficiency %	92
Mean R^2	0.97

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line T25 Using Real-Time PCR - Validation Report and Protocol. EUR 21826 EN. 2005. JRC32123 (ISBN 92-79-00107-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-ACAAGCGTGTCGTGCTCCAC-3'
Target element	Insert
Primer Reverse	5'-GACATGATACTCCTTCCACCG-3'
Target element	3'-host genome
Amplicon length	102 bp
Probe	5'-FAM-TCATTGAGTCGTTCCGCCATTGTCG-TAMRA-3'
Target element	DNA sequence within the 3'-IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-FAM-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Target element	alcohol dehydrogenase 1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event TC1507

1. GENERAL INFORMATION

Target genetic element	Integration border region (IBR) between the insert of maize event TC1507 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/010

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event TC1507
Tested GM events	
Event Name	TC 1507
Unique Identifier	DAS-01507-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 12 blind samples representing six GM levels, namely 0%, 0.1%, 0.5%, 0.9%, 2.0 % and 5.0% of maize event TC1507 DNA in non-GM maize DNA. In addition the laboratories received four calibration samples, two negative DNA target controls consisting of Bt 176 and non-GM maize DNA, an amplification reagent control, reaction reagents, primers and probes for the high-mobility-group A (*hmgA*) reference gene and for the TC 1507 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	not reported	**LOD Absolute**	1.25
LOQ Relative	≤ 0.08%	**LOQ Absolute**	≤ 10

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	0.90%	2%	5.0%	0%
Mean Value (%)	0.11%	0.48%	0.93%	2%	5.4%	0%
RSD_r (%)	18%	12%	7.7%	8.5%	14%	
RSD_R (%)	20%	15%	10%	21%	22%	
Bias %	6.0%	-4.0%	3.7%	-1.7%	8.4%	

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.4
Mean PCR Efficiency %	97	95
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Foti N, Price S, Paoletti C, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line TC1507 Using Real-Time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Maize TC 1507. EUR 21828 EN. 2005. JRC32120 (ISBN 92-79-00109-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TAGTCTTCGGCCAGAATGG-3'
Target element	not specified
Primer Reverse	5'-CTTTGCCAAGATCAAGCG-3'
Target element	not specified
Amplicon length	58 bp
Probe	5'-FAM-TAACTCAAGGCCCTCACTCCG-TAMRA-3'
Probe Name	MaiY-S1
Target element	DNA sequence in the IBR

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Probe Name	Mhmg-probe
Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (including ROX™)	1x	PCR Buffer 10x (including ROX™)	1x
Primer Fw	0,30 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,15 µmol/L	Probe	0,18 µmol/L
$MgCl_2$	5,5 mmol/L	$MgCl_2$	4,5 mmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 U	AmpliTaq Gold® DNA Polymerase	1,0 U
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize event 3272

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of event 3272 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ZM/019

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event 3272 leaves
Tested GM events	
Event Name	3272
Unique Identifier	SYN-E3272-5
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 5% and 8% of maize event 3272 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenase 1 (*adh1)* reference system and for the 3272 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	< 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.40%	0.90%	5.0%	8.0%
Mean Value (%)	0.08%	0.37%	0.79%	4.7%	7.4%
RSD_r (%)	16%	11%	7.1%	6.7%	15%
RSD_R (%)	16%	12%	10%	9.9%	17%
Bias %	-9.6%	-8.5%	-12%	-6.8%	-8.2%

	GMO Target
Mean Slope	-3.3
Mean PCR Efficiency %	102
Mean R^2	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Foti N, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Maize Event 3272 Using Real-time PCR - Validation Report and Protocol. EUR 23645 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48922 (ISBN 176-92-79-71045-0)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TCATCAGACCAGATTCTCTTTTATGG-3'
Target element	5'-host genome
Primer Reverse	5'-CGTTTCCCGCCTTCAGTTTA-3'
Target element	insert
Amplicon length	95 bp
Probe	5'-FAM-ACTGCTGACGCGGCCAAACACTG-TAMRA-3'
Probe Name	ES3272-P
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-VIC-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Probe Name	Zm adh1-P
Target element	alcohol dehydrogenase 1 (*adh1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
JumpStart™ Taq ReadyMix™ (Sigma)	1x	JumpStart™ Taq ReadyMix™ (Sigma)	1x
Primer Fw	0,050 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,90 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 250	Template DNA	maximum 250
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of soybean event A2704-12

1. GENERAL INFORMATION

Target genetic element	Junction region containing a 3' bla sequence and an inverted 5' bla sequence
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event A2704
Tested GM events	
Event Name	A2704-12
Unique Identifier	ACS-GM005-3
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received twenty unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2% and 3.3% of soybean event A2704-12 DNA in non-GM soybean DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the lectin (*Le1)* reference gene and for the A2704-12 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.023%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.045%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.0%	3.3%
Mean Value (%)	0.12%	0.42%	0.92%	2.1%	3.5%
RSD_r (%)	13%	8%	11%	6%	9%
RSD_R (%)	16%	9%	16%	10%	14%
Bias %	18%	6%	2%	3%	5%

	GMO Target
Mean Slope	-3.5
Mean PCR Efficiency %	93
Mean R^2	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Charles Delobel C, Larcher S, Grazioli E, Savini C, Van Den Eede G. Event-Specific Method for the Quantification of Soybean Line A2704-12 Using Real-Time PCR- Validation Report and Protocol - Soybean Seeds Sampling and DNA Extraction. EUR 22910 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37483 (ISBN 978-92-79-06928-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCAAAAAAGCGGTTAGCTCCT-3'
Target element	bla
Primer Reverse	5'-ATTCAGGCTGCGCAACTGTT-3'
Target element	pUC19
Amplicon length	64 bp
Probe	5'-FAM-CGGTCCTCCGATCGCCCTTCC-TAMRA-3'
Probe Name	TM031
Target element	DNA sequence in the junction region

Taxon-target(s)

Primer Forward	5'-CACCTTTCTCGCACCAATTGACA-3'
Target element	*Le1*
Primer Reverse	5'-TCAAACTCAACAGCGACGAC-3'
Target element	*Le1*
Amplicon length	105 bp
Probe	5'-FAM-CCACAAACACATGCAGGTTATCTTGG-TAMRA-3'
Probe Name	TM021
Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of soybean event A5547-127

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event A5547-127 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/007

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM soybean event A5547-127 leaves
Tested GM events	
Event name	A5547-127
Unique Identifier	ACS-GM006-4
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.08%, 0.4%, 0.9%, 4.0% and 8.0% of soybean event A5547-127 DNA in non-GM soybean DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the lectin (*Le1*) reference gene and for the A5547-127 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.023%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.08%	0.40%	0.90%	4.0%	8.0%
Mean Value (%)	0.1%	0.44%	0.98%	4.1%	7.7%
RSD_r (%)	8%	7%	10%	5%	6%
RSD_R (%)	16%	16%	11%	9%	10%
Bias %	25%	10%	9%	2%	-4%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	93	94
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Bogni A, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Soybean Line A5547-127 Using Real-time PCR - Validation Report and Protocol. EUR 24240 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56620 (ISBN 978-92-79-14986-3)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCTATTTGGTGGCATTTTTCCA-3'
Target element	5'-host genome
Primer Reverse	5'-CACTGCGGCCAACTTACTTCT-3'
Target element	Insert
Amplicon length	75 bp
Probe	5'-FAM-CCGCAATGTCATACCGTCATCGTTGT-TAMRA-3'
Probe Name	TM058
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CTTTCTCGCACCAATTGACA-3'
Target element	*Le1*
Primer Reverse	5'-TCAAACTCAACAGCGACGAC-3'
Target element	*Le1*
Amplicon length	102 bp
Probe	5'-VIC-CCACAAACACATGCAGGTTATCTTGG-TAMRA-3'
Probe Name	TM021
Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of soybean event 305423

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of soybean event 305423 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/008

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event DP-305423-1 beans
Tested GM events	
Event Name	305423
Unique Identifier	DP-305423-1
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.09%, 0.5%, 0.9%, 2% and 5% of soybean event 305423 DNA in non-GM soybean DNA. In addition the laboratories received four calibration samples, an amplification control sample, primers and probes for the lectin (*Le1*) reference gene and for the 305423 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.50%	0.90%	2.0%	5.0%
Mean Value (%)	0.08%	0.47%	0.98%	1.9%	5.1%
RSD_r (%)	17%	14%	12%	12%	11%
RSD_R (%)	22%	17%	17%	18%	17%
Bias %	-6.3%	-6.8%	8.4%	-3.1%	2.1%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.4
Mean PCR Efficiency %	99	95
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Munaro B, Grazioli E, Savini C, Charles Delobel C, Van Den Eede G. Event-specific Method for the Quantification of Soybean Event DP-305423-1 Using Real-time PCR - Validation Report and Protocol. EUR 24156 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56604 (ISBN 978-92-79-14881-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CGTGTTCTCTTTTTGGCTAGC-3'
Target element	Insert
Primer Reverse	5'-GTGACCAATGAATACATAACACAAACTA-3'
Target element	3'-host genome
Amplicon length	93 bp
Probe	5'-FAM-TGACACAAATGATTTTCATACAAAAGTCGAGA-TAMRA-3'
Probe Name	DP305-p
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	*Le1*
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	*Le1*
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA-3'
Probe Name	Lec
Target element	lectin (*Le1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,80 µmol/L	Primer Fw	0,55 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,55 µmol/L
Probe	0,22 µmol/L	Probe	0,10 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	Maximum 100	Template DNA	maximum 100
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of soybean event 356043

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event 356043 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/009

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event 356043 beans
Tested GM events	
Event Name	356043
Unique Identifier	DP-356043-5
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.09%, 0.5%, 0.9%, 2% and 5% of soybean event 356043 DNA in non-GM soybean DNA. In addition the laboratories received four calibration samples, an amplification control sample, primers and probes for the lectin (*Le1*) reference gene and for the 356043 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	**0.09%**	**0.50%**	**0.90%**	**2%**	**5.0%**
Mean Value (%)	0.10%	0.55%	0.95%	2%	4.9%
RSD_r (%)	15%	16%	9.9%	10%	8.9%
RSD_R (%)	18%	17%	12%	16%	11%
Bias %	11%	10%	5.5%	-0.15%	-2.9%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.4
Mean PCR Efficiency %	96	98
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Munaro B, Grazioli E, Savini C, Charles Delobel C, Van Den Eede G. Event-specific Method for the Quantification of Soybean Event DP-356043-5 Using Real-time PCR - Validation Report and Protocol. EUR 24157 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56605 (ISBN 978-92-79-14882-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTCGAATAGGCTAGGTTTACGAAAAA-3'
Target element	5'-host genome
Primer Reverse	5'-TTTGATATTCTTGGAGTAGACGAGAGTGT-3'
Target element	Insert
Amplicon length	99 bp
Probe	5'-FAM-CTCTAGAGATCCGTCAACATGGTGGAGCAC-TAMRA-3'
Probe Name	DP356-p
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	*Le1*
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	*Le1*
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA-3'
Probe Name	lec
Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,75 µmol/L	Primer Fw	0,65 µmol/L
Primer Rev	0,75 µmol/L	Primer Rev	0,65 µmol/L
Probe	0,20 µmol/L	Probe	0,18 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	Maximum 100	Template DNA	maximum 100
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of soybean event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	Junction region between the chloroplast-transit-signal peptide (CTP) sequence from Petunia hybrida *epsps* gene and the CP4 *epsps* gene (CP4-EPSPS) from *Agrobacterium tumefasciens*
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/001

2. VALIDATION DATA

Collaborative trial coordinator	National Food Research Institute of Japan (NFRI)
Test material applied in collaborative trial	Soybean flour
Materials used for calibration/controls	plasmid pMulSL2 (Fasmac Co, Ltd. and Nippon Gene Co.)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of soya bean powder derived from the soybean event GTS 40-3-2 and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

LOD Relative	0.1%	**LOD Absolute**	20 HGE
LOQ Relative	0.1%	**LOQ Absolute**	20 HGE

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.50%**	**1.0%**	**5.0%**	**10%**
Mean Value (%)	0.11%	0.57%	1.2%	5.8%	12%
RSD_r (%)	13%	12%	11%	7.6%	8.5%
RSD_R (%)	13%	16%	14%	12%	11%
Bias %	8.1%	14%	16%	15%	17%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The absolute LOD and LOQ values were not determined in this collaborative trial.

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoda, and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Genetically Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC International, Vol. 85, No. 5, p. 1119-1126

IISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CCTTTAGGATTTCAGCATCAGTGG-3'
Target element	*CTP*
Primer Reverse	5'-GACTTGTCGCCGGGAATG-3'
Target element	*CP4-EPSPS*
Amplicon length	121 bp
Probe	5'-FAM-CGCAACCGCCCGCAAATCC-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCA-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTT-3'
Target element	*Le1*
Amplicon length	118 bp
Probe	5'-FAM-AGCTTCGCCGCTTCCTTCAACTTCAC-TAMRA-3'
Target element	lectin *(Le1)* gene
Plasmid Standard	Yes
Plasmid Standard Name	plasmid pMulSL2

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Template DNA	50 ng	Template DNA	50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing & Extension	59°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of soybean event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	Junction region between the chloroplast-transit-signal peptide (CTP) sequence from Petunia hybrida *epsps* gene and the *CP4 epsps* gene from *Agrobacterium tumefasciens*
PCR Assay	Duplex real-time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/002

2. VALIDATION DATA

Collaborative trial coordinator	Central Science Laboratory, York
Test material applied in collaborative trial	Soy flour
Materials used for calibration/controls	IRMM-410R (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

2.1 Collaborative Trial Description (not verified)

All participants received 10 encoded samples of soya flour (4 blind duplicates at split levels) containing 0%, 0.5%, 0.7%, 1.6%, 2%, and 3.9% (w/w) genetically modified soybean event GTS 40-3-2. Two DNA extracts from each material were to be prepared and assayed using primers and probe sets specific for the lectin (*Le1*) as the endogenous control and the GM insert as the target. The percentage of genetically modified soybean in the samples was calculated by using a matrix-matched standard curve.

2.2 Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

- Values determined in the collaborative trial

Test Level (%)	0.5%	1.6%	2%	3.9%	0%
Specificity (%)	-	-	-	-	100%
Mean Value (%)	0.56%	1.6%	2%	3.9%	-
RSD_r (%)	14%	19%	9.3%	10%	-
RSD_R (%)	27%	34%	23%	20%	-
Bias (%)	n.a.	n.a.	n.a.	n.a.	-

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
Data received for the split level and consisting of samples of 0.5% and 0.7% GM soya in soya flour were combined in the reported statistical analysis. The corresponding values are here represented under level 0.5%.

3. REFERENCES

H. Hird, J. Powell, M-L Johnson, and S Oehlschlager (2003) 'Determination of Percentage of RoundUp Ready_ Soya in Soya Flour Using Real-Time Polymerase Chain Reaction:Interlaboratory Study' Journal of AOAC International, Vol. 86, No. 1, p. 66-71

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer forward	5'-GGATTTCAGCATCAGTGGCTACA-3'
Target element	*CTP4*
Primer reverse	5'-CCGGAAAGGCCAGAGGAT-3'
Target element	*CP4-EPSPS*
Amplicon length	88 bp
Probe	5'-FAM-CCGGCTGCTTGCACCGTGAAG-TAMRA-3'
Target element	DNA sequence in the junction region

Taxon-target(s)

Primer forward	5'-TGGTCGCGCCCTCTACTC-3'
Target element	*Le1*
Primer reverse	5'-GGCGAAGCTGGCAACG-3'
Target element	*Le1*
Amplicon length	70 bp
Probe	5'-VIC-CTACCGGTTTCTTTGTCCCAAATGTGGAT-TAMRA-3'
Target element	lectin (*Le1*) gene

5. PCR REACTION SETUP

GM-target(s) and taxon-target(s)

Reagent	Final Concentration
TaqMan® PCR Master Mix	1x
GM-target primer forward	0,90 µmol/L
GM-target primer reverse	0,90 µmol/L
GM-target probe	0,125 µmol/L
Taxon-target primer forward	0,30 µmol/L
Taxon-target primer reverse	0,30 µmol/L
Taxon-target probe	0,120 µmol/L
Deionized sterile water	#
Template DNA	5 µL
Final volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/initial denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing and extension	60°C	60"	
Denaturing, annealing and extension			60

Quantitative PCR method for detection of soybean event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	Junction region between the Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and the chloroplast-transit-signal peptide (CTP) sequence from the Petunia hybrida *epsps* gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/003

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Soybean CRM flour
Materials used for calibration/controls	CRM IRMM-410 (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

2.1 Collaborative Trial Description

All laboratories received four DNA standards, six encoded samples consisting of soybean meal containing 0.1%, 0.5%, 1%, 2%, 5% soybean event GTS 40-3-2 (RRS) and one sample containing texturized vegetable protein (TVP) supplied with 2% RRS. Each laboratory had to perform two independent DNA extractions.

2.2 Method Performance

LOD Relative	not reported	**LOD Absolute**	5 HGE
LOQ Relative	not reported	**LOQ Absolute**	≤50 HGE

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.50%**	**1%**	**2.0%**	**5.0%**
Mean Value (%)	0.11%	0.49%	1%	2.3%	5.1%
RSD_r (%)	33%	24%	21%	11%	10%
RSD_R (%)	44%	27%	28%	32%	27%
Bias (%)	not reported	not reported	not reported	not reported	not reported

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency (%)	not reported	not reported
Mean R^2	not reported	not reported

Comment
The LOD and LOQ values were not reported in this collaborative trial.

3. REFERENCES

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CATTTGGAGAGGACACGCTGA-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-GAGCCATGTTGTTAATTTGTGCC-3'
Target element	*CTP4*
Amplicon length	74 bp
Probe	5'-FAM-CAAGCTGACTCTAGCAGATCTTTC-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	*Le1*
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	*Le1*
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA-3'
Target element	lectin *(Le1)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan buffer A (with ROX™)	1x	TaqMan buffer A (with ROX™)	1x
$MgCl_2$	4,5 mmol/L	$MgCl_2$	4,5 mmol/L
AmpliTaq Gold® DNA Polymerase	1,25 U	AmpliTaq Gold® DNA Polymerase	1,25 U
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
Uracil-N-glycosylase (UNG)	0,5 U	Uracil-N-glycosylase (UNG)	0,5 U
Primer Fw	0,60 µmol/L	Primer Fw	0,60 µmol/L
Primer Rev	0,60 µmol/L	Primer Rev	0,60 µmol/L
Probe	0,125 µmol/L	Probe	0,120 µmol/L
Deionized sterile water	#	Deionized sterile water	#
Template DNA	1,7-108 ng	Template DNA	1,7-108 ng
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of soybean event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event GTS 40-3-2 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/005

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event GTS 40-3-2 seeds
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 4.0% and 8.0% of soybean event GTS 40-3-2 DNA in non-GM soybean DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the lectin (*Le1)* reference gene and for the GTS 40-3-2 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	4%	8.0%
Mean Value (%)	0.09%	0.36%	0.86%	4%	9.1%
RSD_r (%)	29%	26%	22%	28%	29%
RSD_R (%)	40%	30%	28%	32%	32%
Bias %	-6%	-11%	-4%	0%	14%

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.3
Mean PCR Efficiency %	92	95
Mean R^2	0.98	0.98

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Munaro B, Larcher S, Grazioli E, Charles Delobel C, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Soybean Line 40-3-2 Using Real-time PCR - Validation Report and Protocol - Report on the Validation of a DNA Extraction Method for Soybean Seeds. EUR 23086 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC42837 (ISBN 978-92-79-08179-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TTCATTCAAAATAAGATCATACATACAGGTT-3'
Target element	5'-host genome
Primer Reverse	5'-GGCATTTGTAGGAGCCACCTT-3'
Target element	Insert
Amplicon length	84 bp
Probe	5'-FAM-CCTTTTCCATTTGGG-MGBNFQ-3'
Probe Name	40-3-2 AP
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	*Le1*
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	*Le1*
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA-3'
Probe Name	lecP
Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s) and taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1	95°C	600"	1
Denaturation	95°C	15"		95°C	15"	
Annealing & Extension	55°C	60"		60°C	60"	
Denaturing, Annealing & Extension			45			45

Quantitative PCR method for detection of soybean event MON 89788

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event MON 89788 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GM/006

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event MON 89788 seeds
Tested GM events	
Event Name	MON 89788
Unique Identifier	MON-89788-1
Crop Name	*Glycine max* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 4.0% and 8.0% of soybean event MON 89788 DNA in non-GM soybean DNA. In addition, the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the lectin (*Le1)* reference gene and for the MON 89788 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	4.0%	8.0%
Mean Value (%)	0.09%	0.38%	0.89%	4.4%	8.2%
RSD_r (%)	16%	22%	15%	13%	12%
RSD_R (%)	20%	25%	18%	16%	12%
Bias %	-14%	-5%	-0.9%	11%	2.8%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.3
Mean PCR Efficiency %	98	100
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Bogni A, Pinski G, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Soybean Line MON 89788 Using Real-time PCR - Validation Report and Protocol. EUR 23653 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48852 (ISBN 978-92-79-11053-5)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TCCCGCTCTAGCGCTTCAAT-3'
Target element	5'-host genome
Primer Reverse	5'-TCGAGCAGGACCTGCAGAA-3'
Target element	Insert
Amplicon length	139 bp
Probe	5'-FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	*Le1*
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	*Le1*
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA-3'
Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event GHB 614

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of cotton event GHB 614 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/006

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM cotton event GHB 614 cotton
Tested GM events	
Event Name	GHB 614
Unique Identifier	BCS-GH002-5
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received twenty unknown samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 2% and 4.5% of cotton event GHB614 DNA in non-GM cotton DNA. In addition participants received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenase C (*adhC)* reference gene and for the GHB 614 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.023%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.40%	0.90%	2.0%	4.5%
Mean Value (%)	0.10%	0.46%	0.97%	2.2%	4.6%
RSD_r (%)	9.4%	15%	6.8%	3.3%	4.1%
RSD_R (%)	12%	17%	8.3%	4.4%	5.1%
Bias %	15%	14%	7.5%	8.8%	2.0%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	94	92
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Savini C, Bogni A, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line GHB 614 Using Real-time PCR - Validation Report and Protocol. EUR 23648 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48918 *(ISBN 978-92-79-11048-1)*

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAAATACACTTGGAACGACTTCGT-3'
Target element	Insert
Primer Reverse	5'-GCAGGCATGCAAGCTTTTAAA-3'
Target element	3'-host genome
Amplicon length	120 bp
Probe	5'-FAM-CTCCATGGCGATCGCTACGTTCTAGAATT-TAMRA-3'
Probe Name	TM072
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-CACATGACTTAGCCCATCTTTGC-3'
Target element	*adhC*
Primer Reverse	5'-CCCACCCTTTTTTGGTTTAGC-3'
Target element	*adhC*
Amplicon length	73 bp
Probe	5'-VIC-TGCAGGTTTTGGTGCCACTGTGAATG-TAMRA-3'
Probe Name	TM012
Target element	alcohol dehydrogenase C (*adhC*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event LLCotton25

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event LLCotton25 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/002

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event LLcotton25
Tested GM events	
Event Name	LLCotton25
Unique Identifier	ACS-GH001-3
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received twenty blind DNA samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3% of cotton event LLCotton25 DNA in non-GM cotton DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenase C (*adhC)* reference gene and for the LLCotton25 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.15%	0.40%	0.9%	2.0%	3.3%
Mean Value (%)	0.17%	0.47%	1.1%	2.2%	3.6%
RSD_r (%)	23%	28%	18%	18%	24%
RSD_R (%)	23%	32%	32%	24%	30%
Bias %	12%	17%	20%	11%	8.1%

	GMO Target
Mean Slope	-3.5
Mean PCR Efficiency %	91
Mean R^2	0.97

Comment
The LOD and LOQ relative values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Grazioli E, Van Den Eede G. Event-Specific Method for the Quantification of Cotton Line "LLCotton25" Using Real-Time PCR - Validation Report and Protocol - Cotton Seeds Sampling and DNA Extraction. EUR 22912 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37488 (ISBN 978-92-79-06932-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAAGGAACTATTCAACTGAG-3'
Target element	5'-host genome
Primer Reverse	5'-CAGATTTTTGTGGGATTGGAATTC-3'
Target element	Insert
Amplicon length	79 bp
Probe	5'-FAM-CTTAACAGTACTCGGCCGTCGACGC-TAMRA-3'
Probe Name	TM018
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CACATGACTTAGCCCATCTTTGC-3'
Target element	*adhC*
Primer Reverse	5'-CCCACCCTTTTTTGGTTTAGC-3'
Target element	*adhC*
Amplicon length	73 bp
Probe	5'-FAM-TGCAGGTTTTGGTGCCACTGTGAATG-TAMRA-3'
Probe Name	TM012
Target element	alcohol dehydrogenase C (*adhC*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event MON 531

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event MON 351 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event MON 531 seeds
Tested GM events	
Event Name	MON 531
Unique Identifier	MON-00531-6
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.5%, 0.9%, 2.5% and 6.0% of cotton event MON 531 DNA in non-GM cotton DNA. In addition, the laboratories received five calibration samples, reaction reagents, primers and probes for the acyl carrier protein 1 (*acp1*) reference gene and for the MON 531 specific system. Two separated plates were run due to the difference in the annealing temperature between the two systems used. On each plate, the samples were analysed either for the MON 531 specific system or the *acp1* reference system. In total, two plates were run per participating laboratory and four replicates for each GM level were analysed.

Method Performance

LOD Relative	≤ 0.05%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	0.9%	2.5%	6.0%
Mean Value (%)	0.08%	0.36%	0.7%	2.3%	6.2%
RSD_r (%)	34%	22%	21%	15%	21%
RSD_R (%)	43%	31%	32%	24%	28%
Bias %	-22%	-28%	-22%	-6.4%	2.5%

	GMO Target	Taxon Target
Mean Slope	-2.9	-3.1
Mean PCR Efficiency %	80	90
Mean R^2	0.97	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Bogni A, Foti N, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line MON 531 Using Real-time PCR - Validation Report and Protocol. EUR 23651 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48906 (ISBN 978-92-79-11051-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TCCCATTCGAGTTTCTCACGT-3'
Target element	Insert
Primer Reverse	5'-AACCAATGCCACCCCACTGA-3'
Target element	5'-host genome
Amplicon length	72 bp
Probe	5'-FAM-TTGTCCCTCCACTTCTTCTC-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-ATTGTGATGGGACTTGAGGAAGA-3'
Target element	*acp1*
Primer Reverse	5'-CTTGAACAGTTGTGATGGATTGTG-3'
Target element	*acp1*
Amplicon length	76 bp
Probe	5'-FAM-ATTGTCCTCTTCCACCGTGATTCCGAA-TAMRA-3'
Target element	acyl carrier protein 1 (*acp1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,050 µmol/L	Probe	0,050 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			**Taxon-target(s)**		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1	95°C	600"	1
Denaturation	95°C	15"		95°C	15"	
Annealing & Extension	55°C	60"		60°C	60"	
Denaturing, Annealing & Extension			45			45

Quantitative PCR method for detection of cotton event MON 1445

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event MON 1445 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/003

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event MON 1445 seeds
Tested GM events	
Event Name	MON 1445
Unique Identifier	MON-01445-2
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.5%, 0.9%, 2.5% and 6.0% of cotton event MON 1445 DNA in non-GM cotton DNA. In addition, the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the acyl carrier protein 1 (*acp1*) reference gene and for the MON 1445 specific system. Four replicates for GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	0.90%	2.5%	6.0%
Mean Value (%)	0.14%	0.62%	0.94%	2.8%	6.3%
RSD_r (%)	14%	18%	13%	11%	17%
RSD_R (%)	21%	18%	17%	16%	24%
Bias %	41%	25%	4.7%	11%	5.2%

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.4
Mean PCR Efficiency %	104	101
Mean R^2	0.98	0.98

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial

3. REFERENCES

Mazzara M, Bogni A, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line MON 1445 Using Real-timePCR - Validation Report and Protocol. EUR 23652 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48853 (ISBN 978-92-79-11052-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GGAGTAAGACGATTCAGATCAAACAC-3'
Target element	5'-host genome
Primer Reverse	5'-ATCGACCTGCAGCCCAAGCT-3'
Target element	Insert
Amplicon length	87 bp
Probe	5'-FAM-ATCAGATTGTCGTTTCCCGCCTTCAGTTT-TAMRA-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-ATTGTGATGGGACTTGAGGAAGA-3'
Target element	*acp1*
Primer Reverse	5'-CTTGAACAGTTGTGATGGATTGTG-3'
Target element	*acp1*
Amplicon length	76 bp
Probe	5'-FAM-ATTGTCCTCTTCCACCGTGATTCCGAA-TAMRA-3'
Target element	acyl carrier protein 1 (*acp1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event MON 15985

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of cotton event 15985 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/005

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event MON 15985 seeds
Tested GM events	
Event Name	MON 15985
Unique Identifier	MON-15985-7
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.5% and 6.0% of cotton event MON 15985 DNA in non-GM cotton DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the acyl carrier protein 1 (*acp1*) reference gene and for the MON 15985 specific system. Four replicates for GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.05%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.5%	6%
Mean Value (%)	0.08%	0.33%	0.84%	2.5%	6%
RSD_r (%)	19%	16%	22%	26%	15%
RSD_R (%)	42%	33%	27%	27%	16%
Bias %	-21%	-18%	-7.2%	-0.5%	0.5%

	GMO Target	Taxon Target
Mean Slope	-3.2	-3.3
Mean PCR Efficiency %	110	103
Mean R^2	0.99	0.98

Comment
The relative LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Savini C, Mazzara M, Munaro B, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line MON 15985 Using Real-timePCR - Validation Report and Protocol. EUR 23650 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48908 (ISBN 978-92-79-11050-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTTACTAGATCGGGGATATCC-3'
Target element	Insert
Primer Reverse	5'-AAGGTTGCTAAATGGATGGGA-3'
Target element	3'-host genome
Amplicon length	82 bp
Probe	5'-FAM-CCGCTCTAGAACTAGTGGATCTGCACTGAA-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-ATTGTGATGGGACTTGAGGAAGA-3'
Target element	*acp1*
Primer Reverse	5'-CTTGAACAGTTGTGATGGATTGTG-3'
Target element	*acp1*
Amplicon length	76 bp
Probe	5'-FAM-ATTGTCCTCTTCCACCGTGATTCCGAA-TAMRA-3'
Target element	acyl carrier protein 1 (*acp1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event MON 88913

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event MON 88913 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/007

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event MON 88913 seeds
Tested GM events	
Event Name	MON 88913
Unique Identifier	MON-88913-8
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.09%, 0.3%, 0.9%, 3.0% and 8.0% of cotton event MON 88913 DNA in non-GM cotton DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the acyl carrier protein 1 (*acp1*) reference gene and for the MON 88913 specific system. Four replicates for GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.3%	0.90%	3.0%	8.0%
Mean Value (%)	0.08%	0.3%	0.66%	2.7%	7.4%
RSD_r (%)	13%	10%	13%	11%	12%
RSD_R (%)	25%	12%	21%	16%	12%
Bias %	-16%	0.5%	-27%	-12%	-7.2%

	GMO Target	Taxon Target
Mean Slope	-3.2	-3.3
Mean PCR Efficiency %	105	101
Mean R^2	0.99	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Luque Perez E, Pinski G, Bogni A, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Cotton MON 88913 Using Real-time PCR - Validation Report and Protocol. EUR 24159 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56608 (ISBN 978-92-79-14980-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAAATTACCCATTAAGTAGCCAAATTAC-3'
Target element	5'-host genome
Primer Reverse	5'-GGCTTTGGCTACCTTAAGAGAGTC-3'
Target element	Insert
Amplicon length	94 bp
Probe	5'-FAM-AACTATCAGTGTTTGACTACAT-MGBNFQ-3'
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-ATTGTGATGGGACTTGAGGAAGA-3'
Target element	*acp1*
Primer Reverse	5'-CTTGAACAGTTGTGATGGATTGTG-3'
Target element	*acp1*
Amplicon length	76 bp
Probe	5'-FAM-ATTGTCCTCTTCCACCGTGATTCCGAA-TAMRA-3'
Target element	acyl carrier protein 1 (*acp1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,50 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,10 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event 281-24-236

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of cotton event 281-24-236 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/001a

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM cotton event 281-24-236 x 3006-210-23
Tested GM events	
Event Name	281-24-236 x 3006-210-23
Unique Identifier	DAS-24236-5 x DAS-21023-5
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.0% and 5.5% of cotton event 281-24-236 x 3006-210-23 DNA in non-GM cotton DNA. In addition the laboratories received four calibration samples, a negative control, an amplification control, reaction reagents, primers and probes for the Sinapis Arabidopsis Homolog 7 (*SAH7)* reference gene and for the 281-24-236 specific system. Four replicates for each GM level analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.0%	5.5%
Mean Value (%)	0.11%	0.42%	0.95%	2.2%	5.6%
RSD_r (%)	22%	16%	17%	15%	15%
RSD_R (%)	29%	23%	20%	17%	17%
Bias %	5.3%	3.9%	5.3%	10.0%	2.7%

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.4
Mean PCR Efficiency %	96	94
Mean R^2	1.00	0.99

Comment
The LOD and LOQ relative values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Larcher S, Savini C, Charles Delobel C, Van Den Eede G. Event-Specific Methods for the Quantitation of the Hybrid Cotton Line 281-24-236/3006-210-23 Using Real-Time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Cotton Seeds. EUR 22473 EN. 2006. JRC33249 (ISBN 92-79-031107-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CTCATTGCTGATCCATGTAGATTTC-3'
Target element	Insert
Primer Reverse	5'-GGACAATGCTGGGCTTTGTG-3'
Target element	3'-host genome
Amplicon length	111 bp
Probe	5'-FAM-TTGGGTTAATAAAGTCAGATTAGAGGGAGACAA-TAMRA-3'
Probe Name	281-S2
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-AGTTTGTAGGTTTTGATGTTACATTGAG-3'
Target element	SAH7
Primer Reverse	5'-GCATCTTTGAACCGCCTACTG-3'
Target element	SAH7
Amplicon length	115 bp and 123 bp
Probe	5'-FAM-AAACATAAAATAATGGGAACAACCATGACATGT-TAMRA-3'
Probe Name	Sah7-uni-s1
Target element	Sinapis Arabidopsis Homolog 7 (*SAH7*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer II (10x)	1x	PCR buffer II (10x)	1x
ROX™reference dye	0,7x	ROX™reference dye	0,7x
Tween-20	0,01%	Tween-20	0,01%
Glycerol	0,8%	Glycerol	0,8%
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
$MgCl_2$	5,0 mmol/L	$MgCl_2$	6,0 mmol/L
Primer Fw	0,35 µmol/L	Primer Fw	0,35 µmol/L
Primer Rev	0,45 µmol/L	Primer Rev	0,25 µmol/L
Probe	0,175 µmol/L	Probe	0,175 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 U	AmpliTaq Gold® DNA Polymerase	1,0 U
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 100	Template DNA	maximum 100 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of cotton event 3006-210-23

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event 3006-210-23 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/GH/001b

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM event 281-24-236 x 3006-210-23 cotton
Tested GM events	
Event Name	281-24-236 x 3006-210-23
Unique Identifier	DAS-24236-5 x DAS-21023-5
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.0% and 5.5% of cotton event 281-24-236 x 3006-210-23 DNA in non-GM cotton transgenic genomic DNA. In addition the laboratories received four calibration samples, a negative control, an amplification control, reaction reagents, primers and probes for the *Sinapis Arabidopsis homolog 7 (SAH7)* reference gene and for the 3006-210-23 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.0%	5.5%
Mean Value (%)	0.09%	0.39%	0.91%	2.1%	5.6%
RSD_r (%)	30%	20%	16%	15%	21%
RSD_R (%)	32%	21%	21%	19%	22%
Bias %	-5.6%	-1.4%	0.95%	2.8%	2.5%

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.3
Mean PCR Efficiency %	94	94
Mean R^2	1.00	0.99

Comment
The LOD and LOQ relative values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Larcher S, Savini C, Charles Delobel C, Van Den Eede G. Event-Specific Methods for the Quantitation of the Hybrid Cotton Line 281-24-236/3006-210-23 Using Real-Time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Cotton Seeds. EUR 22473 EN. 2006. JRC33249 ((ISBN 92-79-031107-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AAATATTAACAATGCATTGAGTATGATG-3'
Target element	5'-host genome
Primer Reverse	5'-ACTCTTTCTTTTTCTCCATATTGACC-3'
Target element	Insert
Amplicon length	90 bp
Probe	5'-FAM-TACTCATTGCTGATCCATGTAGATTTCCCG-TAMRA-3'
Probe Name	3006-s2
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-AGTTTGTAGGTTTTGATGTTACATTGAG-3'
Target element	SAH7
Primer Reverse	5'-GCATCTTTGAACCGCCTACTG-3'
Target element	SAH7
Amplicon length	115 bp and 123 bp
Probe	5'-FAM-AAACATAAAATAATGGGAACAACCATGACATGT-TAMRA-3'
Probe Name	Sah7-uni-s1
Target element	IVS of the *Sinapis Arabidopsis homolog 7 (SAH7)* gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer II (10x)	1x	PCR buffer II (10x)	1x
ROX™reference dye	0,7x	ROX™reference dye	0,7x
Tween-20	0,01%	Tween-20	0,01%
Glycerol	0,8%	Glycerol	0,8%
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
$MgCl_2$	6,0 mmol/L	$MgCl_2$	6,0 mmol/L
Primer Fw	0,40 µmol/L	Primer Fw	0,35 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,25 µmol/L
Probe	0,15 µmol/L	Probe	0,175 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 U	AmpliTaq Gold® DNA Polymerase	1,0 U
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 100	Template DNA	maximum 100 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of oilseed rape event GT73

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of oilseed rape event GT73 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/BN/004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Oilseed rape seeds
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM oilseed rape event GT73 seeds
Tested GM events	
Event Name	GT73 (RT73)
Unique Identifier	MON-00073-7
Crop Name	*Brassica napus* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 4.0% and 8.0% of oilseed rape event GT73 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*CruA)* reference gene and for the GT73 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	4.0%	8.0%
Mean Value (%)	0.08%	0.35%	0.85%	4.2%	8.4%
RSD_r (%)	23%	17%	17%	14%	14%
RSD_R (%)	28%	24%	19%	17%	16%
Bias %	-25%	-13%	-6%	5.8%	4.5%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.4
Mean PCR Efficiency %	95	95
Mean R^2	0.99	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-Specific Method for the Quantification of Oilseed Rape Line RT73 Using Real-Time PCR - Validation Report and Protocol - Seeds Sampling and DNA Extraction of Oilseed Rape. EUR 22918 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37550 (ISBN 978-92-79-06935-2)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CCATATTGACCATCATACTCATTGCT-3'
Target element	Insert
Primer Reverse	5'-GCTTATACGAAGGCAAGAAAAGGA-3'
Target element	3'-host genome
Amplicon length	108 bp
Probe	5'-FAM-TTCCCGGACATGAAGATCATCCTCCTT-TAMRA-3'
Probe Name	RT73 probe
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-GGCCAGGGTTTCCGTGAT-3'
Target element	*cruA*
Primer Reverse	5'-CCGTCGTTGTAGAACCATTGG-3'
Target element	*cruA*
Amplicon length	101 bp
Probe	5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3'
Probe Name	TM003
Target element	cruciferin A (*cruA*) gene

5. PCR REACTIONS SETUP

GM-target(s) Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,05 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of oilseed rape event Ms8

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of oilseed rape event Ms8 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/BN/002

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM oilseed rape event Ms8 seeds
Tested GM events	
Event Name	Ms8
Unique Identifier	ACS-BN005-8
Crop Name	*Brassica napus* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 1.8% and 3.6% of oilseed rape event Ms8 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*cruA)* reference gene and for the Ms8 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	1.8%	3.6%
Mean Value (%)	0.11%	0.39%	0.89%	1.8%	3.3%
RSD_r (%)	22%	18%	14%	17%	11%
RSD_R (%)	23%	21%	14%	23%	17%
Bias %	7.4%	-3.5%	-1.0%	-1.0%	-7.5%

	GMO Target
Mean Slope	-3.4
Mean PCR Efficiency %	92
Mean R^2	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Bogni A, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Oilseed Rape Line Ms8 Using Real-time PCR - Validation Report and Protocol- Seeds Sampling and DNA Extraction of Oilseed Rape. EUR 22917 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37545 (ISBN 978-92-79-06934-5)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTTAGAAAAAGTAAACAATTAATATAGCCGG-3'
Target element	Insert
Primer Reverse	5'-GGAGGGTGTTTTTGGTTATC-3'
Target element	3'-host genome
Amplicon length	130 bp
Probe	5'-FAM-AATATAATCGACGGATCCCCGGGAATTC-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-GGCCAGGGTTTCCGTGAT-3'
Target element	*cruA*
Primer Reverse	5'-CCGTCGTTGTAGAACCATTGG-3'
Target element	*cruA*
Amplicon length	101 bp
Probe	5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3'
Target element	cruciferin A (*cruA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	Maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of oilseed rape event Rf3

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of oilseed rape event Rf3 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/BN/003

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM oilseed rape event Rf3 seeds
Tested GM events	
Event Name	Rf3
Unique Identifier	ACS-BN003-6
Crop Name	*Brassica napus* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 1.8% and 3.6% of oilseed rape event Rf3 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*cruA)* reference gene and for the Ms8 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	1.8%	3.6%
Mean Value (%)	0.11%	0.42%	0.94%	1.8%	3.4%
RSD_r (%)	13%	12%	14%	12%	13%
RSD_R (%)	13%	15%	23%	13%	19%
Bias %	6.9%	4.4%	4.5%	-2.5%	-5.2%

	GMO Target
Mean Slope	-3.6
Mean PCR Efficiency %	89
Mean R^2	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Savini C, Bogni A, Mazzara M, Van Den Eede G. Event-Specific Method for the Quantification of Oilseed Rape Line Rf3 Using Real-time PCR - Validation Report and Protocol - Seeds Sampling and DNA Extraction. EUR 22930 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC3754 (ISBN 978-92-79-06985-7)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CATAAAGGAAGATGGAGACTTGAG-3'
Target element	Insert
Primer Reverse	5'-AGCATTTAGCATGTACCATCAGACA-3'
Target element	3'-host genome
Amplicon length	139 bp
Probe	5'-FAM-CGCACGCTTATCGACCATAAGCCCA-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-GGCCAGGGTTTCCGTGAT-3'
Target element	*cruA*
Primer Reverse	5'-CCGTCGTTGTAGAACCATTGG-3'
Target element	*cruA*
Amplicon length	101 bp
Probe	5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3'
Target element	cruciferin A (*cruA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of oilseed rape event T45

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of oilseed rape event T45 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/BN/001

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non GM and GM event T45 oilseed rape
Tested GM events	
Event Name	T45 (HCN28)
Unique Identifier	ACS-BN008-2
Crop Name	*Brassica napus* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 1.8 % and 3.6% of oilseed rape event T45 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*CruA)* reference gene and for the T45 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤0.045%	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤0.09%	0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	1.8%	3.6%
Mean Value (%)	0.09%	0.37%	0.88%	1.8%	3.6%
RSD_r (%)	16%	22%	17%	11%	17%
RSD_R (%)	26%	23%	20%	20%	25%
Bias %	-11%	-7.8%	-1.7%	-3.0%	-1.3%

	GMO Target
Mean Slope	not reported
Mean PCR Efficiency %	not reported
Mean R^2	not reported

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Bogni A, Mazzara M, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Oilseed Rape Line T45 Using Real-time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Oilseed Rape. EUR 22357 EN. 2006. JRC34761 (ISBN 92-79-02987-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAATGGACACATGAATTATGC-3'
Target element	5'-host genome
Primer Reverse	5'-GACTCTGTATGAACTGTTCGC-3'
Target element	insert
Amplicon length	123 bp
Probe	5'-FAM-TAGAGGACCTAACAGAACTCGCCGT-TAMRA-3'
Probe Name	TM026
Target element	DNA sequence in the 5'-IBR

Taxon-target(s)

Primer Forward	5'-GGCCAGGGTTTCCGTGAT-3'
Target element	cruA
Primer Reverse	5'-CCGTCGTTGTAGAACCATTGG-3'
Target element	cruA
Amplicon length	101 bp
Probe	5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3'
Probe Name	TM003
Target element	cruciferin A (*cruA*) gene

5. PCR REACTIONS SETUP

GM-target(s) Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of potato event EH92-527-1

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of potato event EH92-527-1 and the host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ST/001

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM potato event EH92-527-1
Tested GM events	
Event Name	EH92-527-1
Unique Identifier	BPS-25271-9
Crop Name	*Solanum tuberosum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.2% and 5.5% of potato event EH92-527-1 DNA in non-GM potato DNA. In addition the laboratories received four calibration samples, an amplification reagent control, reaction reagents, primers and probes for the UDP-glucose pyrophosphorylase *(UGPase)* reference gene and for the EH92-527-1 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	not reported	**LOD Absolute**	0.625
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.40%**	**0.90%**	**2.2%**	**5.5%**
Mean Value (%)	0.11%	0.42%	0.97%	2.3%	5.7%
RSD_r (%)	12%	12%	10%	10%	10%
RSD_R (%)	16%	14%	13%	15%	12%
Bias %	4.9%	5.1%	8.2%	4.2%	4.3%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.3
Mean PCR Efficiency %	96	95
Mean R^2	1.00	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

3. REFERENCES

Savini C, Foti N, Mazzara M, Charles Delobel C, Van Den Eede G. Event-specific Method for the Quantification of Event EH92-527-1 Potato Using Real-time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Potato. EUR 22358 EN. 2006. JRC34758 (ISBN 92-79-02988-6)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTGTCAAAACACAATTTACAGCA-3'
Target element	Insert
Primer Reverse	5'-TCCCTTAATTCTCCGCTCATGA-3'
Target element	3'-host genome
Amplicon length	134 bp
Probe	5'-FAM-AGATTGTCGTTTCCCGCCTTCAGTT-TAMRA-3'
Probe Name	St527-S2
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-GGACATGTGAAGAGACGGAGC-3'
Target element	UGPase
Primer Reverse	5'-CCTACCTCTACCCCTCCGC-3'
Target element	UGPase
Amplicon length	88 bp
Probe	5'-FAM-CTACCACCATTACCTCGCACCTCCTCA-TAMRA-3'
Probe Name	UGP-sf1 probe
Target element	UDP-glucose pyrophosphorylase (*UGPase*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer II (10x)	1x	PCR buffer II (10x)	1x
ROX™ reference dye	1x	ROX™ reference dye	1x
Tween-20	0,01%	Tween-20	0,01%
Glycerol	0,8%	Glycerol	0,8%
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
$MgCl_2$	4 mmol/L	$MgCl_2$	5,5 mmol/L
Primer Fw	0,30 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,40 µmol/L
Probe	0,16 µmol/L	Probe	0,20 µmol/L
AmpliTaq Gold® DNA Polymerase	0,04 U/µL	AmpliTaq Gold® DNA Polymerase	0,04 U/µL
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of rice event LLRICE62

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of rice event LLRICE62 and the rice host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/OS/002

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM rice event LLRICE62
Tested GM events	
Event Name	LLRICE62
Unique Identifier	ACS-OS002-5
Crop Name	*Oryza sativa* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3% of rice event LLRICE62 DNA in non-GM rice DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the phospholipase D (*PLD)* reference gene and for the LLRICE62 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.15%	0.40%	0.90%	2.0%	3.3%
Mean Value (%)	0.13%	0.37%	0.84%	1.9%	3.2%
RSD_r (%)	21%	12%	11%	9.8%	12%
RSD_R (%)	22%	14%	17%	12%	15%
Bias %	-11%	-7.4%	-7.1%	-3.2%	-2.4%

	GMO Target
Mean Slope	-3.3
Mean PCR Efficiency %	98
Mean R^2	0.99

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-specific Method for the Quantitation of Rice Line LLRICE62 Using Real-time PCR -Validation Report and Protocol - Sampling and DNA Extraction of Rice. EUR 22490 EN. 2006. JRC34091 (ISBN 92-79-03129-5)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AGCTGGCGTAATAGCGAAGAGG-3'
Target element	Insert
Primer Reverse	5'-TGCTAACGGGTGCATCGTCTA-3'
Target element	3'-host genome
Amplicon length	88 bp
Probe	5'-FAM-CGCACCGATTATTTATACTTTTAGTCCACCT-TAMRA-3'
Probe Name	TM019
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-TGGTGAGCGTTTTGCAGTCT-3'
Target element	PLD
Primer Reverse	5'-CTGATCCACTAGCAGGAGGTCC-3'
Target element	PLD
Amplicon length	68 bp
Probe	5'-FAM-TGTTGTGCTGCCAATGTGGCCTG-TAMRA-3'
Probe Name	TM013
Target element	phospholipase D (*PLD*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of sugar beet event H7-1

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of sugar beet event H7-1 and the sugar beet host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/BV/001

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM sugarbeet event H7-1 seeds
Tested GM events	
Event Name	H7-1
Unique Identifier	KM-000H71-4
Crop Name	*Beta vulgaris* L.

Collaborative Trial Description

The participants received twenty unknown samples representing five GM levels, namely 0.1%, 0.5%, 0.9%, 2.0% and 5% of sugar beet event H7-1 DNA in non-GM sugar beet DNA. In addition the laboratories received four calibration samples, an amplification reagent control, reaction reagents, primers and probes for the glutamine synthase (*GS*) reference gene and the H7-1 sugar beet specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	not reported	**LOD Absolute**	10
LOQ Relative	≤ 0.045%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	0.90%	2.0%	5.0%
Mean Value (%)	0.09%	0.55%	0.96%	2.2%	5.5%
RSD_r (%)	16%	14%	18%	15%	12%
RSD_R (%)	20%	16%	18%	16%	13%
Bias %	-7.4%	10%	6.3%	9.1%	10%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	90	93
Mean R^2	0.98	1.00

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Foti N, Savini C, Van Den Eede G. Event-Specific Method for the Quantitation of Sugarbeet Line H7-1 Using Real-Time PCR - Validation Report and Protocol. EUR 22134 EN. 2006. JRC32190 (ISBN 92-79-01536-2)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGGGATCTGGGTGGCTCTAACT-3'
Target element	5'-host genome
Primer Reverse	5'-AATGCTGCTAAATCCTGAG-3'
Target element	Insert
Amplicon length	108 bp
Probe	5'-FAM-AAGGCGGGAAACGACAATCT-TAMRA-3'
Probe Name	ZRH7
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-GACCTCCATATTACTGAAAGGAAG-3'
Target element	GS
Primer Reverse	5'-GAGTAATTGCTCCATCCTGTTCA-3'
Target element	GS
Amplicon length	118 bp
Probe	5'-FAM-CTACGAAGTTTAAAGTATGTGCCGCTC-TAMRA-3'
Probe Name	GluD1
Target element	glutamine synthase (*GS*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer I (10x)	1x	PCR buffer I (10x)	1x
ROX™ reference dye	1,0 µmol/L	ROX™ reference dye	1,0 µmol/L
$MgCl_2$	7,0 mmol/L	$MgCl_2$	5,0 mmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
Primer Fw	0,40 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,10 µmol/L	Probe	0,10 µmol/L
AmpliTaq Gold® DNA Polymerase	0,04 U/µL	AmpliTaq Gold® DNA Polymerase	0,04 U/µL
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 125	Template DNA	maximum 125
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ELE/001

2. VALIDATION DATA

Collaborative trial coordinator	French National Standardization Association (AFNOR)
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	Genomic DNA
Tested GM events	
Event Name	Bt 176
Unique Identifier	SYN-EV176-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

All 10 laboratories quantified the presence of the CaMV P-35S target in DNA extracts from maize event Bt 176. Two types of extracts were provided to participants: a set of 8 calibration samples containing 0%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, and 2% of Bt 176 DNA and 2 blind samples. All analyses were performed in duplicate at two final DNA concentrations (200ng and 20 ng).

Method Performance

LOD Relative	≤0.01%	**LOD Absolute**	<2 HGE
LOQ Relative	≤0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	**0.1%**	**0.50%**
Mean Value (%)	0.1%	0.53%
RSD_r (%)	not reported	not reported
RSD_R (%)	18%	15%
Bias (%)	not reported	not reported

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The reported LOD and LOQ were not assessed in this collaborative trial. The genomic DNA extracts were prepared by the ring-trial coordinator from Bt 176 maize flour and non-GM counterpart provided by SyngentaSeeds AG.

3. REFERENCES

M. Feinberg, S. Fernandez, S. Cassard, C. Charles-delobel and Y. Bertheau (2005) "Quantitation of 35S Promoter in Maize DNAExtracts from Genetically Modified Organisms Using Real-Time Polymerase Chain Reaction, Part 2: Interlaboratory Study" Journal of AOAC International: Vol. 88, no. 2, p. 558-573

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CGTCTTCAAAGCAAGTGGATTG-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-TCTTGCGAAGGATAGTGGGATT-3'
Target element	*CaMV P-35S*
Amplicon length	79 bp
Probe	5'-FAM-TCTCCACTGACGTAAGGGATGACGCA-TAMRA-3'
Target element	CaMV 35S promoter

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
AmpErase® UNG	0,25 U
$MgCl_2$	3,5 mmol/L
Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L
Probe	200 µmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L
dUTP	400 µmol/L
AmpliTaq Gold® DNA Polymerase	0,625 U
Template DNA	200 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			50

Quantitative PCR method for detection of phosphinothricin N-acetyltransferase gene

1. GENERAL INFORMATION

Target genetic element	Phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ELE/002

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Plasmid DNA
Tested GM events	
Event Name	T25
Unique Identifier	ACS-ZM003-2
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 24 unknown samples of maize wild-type DNA spiked with genetically modified maize event T25 DNA. The concentration levels tested were 0.1%, 0.6%, 0.8%, 1.0%, 1.2%, 1.5% w/w of GM maize. Each sample was analyzed in duplicate on the same PCR plate. For the quantification the participants used a plasmid-based standard curve system, a pGEM vector containing the synthetic phosphinothricin N-acetyltransferase (*pat)* gene and an unrelated 40 nt insertion not present in the synthetic *pat* gene of the maize event T25. The primer pair was designed to target a fragment from the plasmid standard different in size from the GMO target event. The two different amplicons were then detected with a pat-specific probe and a pGEM -specific spike probe.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	≤0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False Positives	0%
False Negatives	not reported

Test Level (%)	**0.10%**	**0.60%**	**0.80%**	**1.00%**	**1.2%**	**1.5%**
Mean Value (%)	0.11%	0.62%	0.83%	0.97%	1.2%	1.5%
RSD_r (%)	16%	12%	10%	12%	13%	8.7%
RSD_R (%)	16%	20%	18%	26%	23%	21%
Bias (%)	not reported	not reported	not reported	not reported	not reported	not reported

	GMO Target	**Control Target**
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	1.00	1.00

Comment
The reported LOD and LOQ were not determined in this collaborative trial.

3. REFERENCES

F. Weighardt, C. Barbati, C. Paoletti, M. Querci, S. Kay, M. De Beuckeleer, and G. Van den Eede (2004) 'Real-Time Polymerase Chain Reaction-Based Approach for Quantification of the *pat* Gene in the T25 *Zea mays* Event' Journal OF AOAC International, Vol. 87, No. 6, p. 1342-1355

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TTGAGGGTGTTGTGGCTGGTA-3'
Target element	*pat*
Primer Reverse	5'-TGTCCAATCGTAAGCGTTCCT-3'
Target element	*pat*
Probe 1	5'-FAM-CTTCCAGGGCCCAGCGTAAGCA-TAMRA-3'
Target element	phosphinothricin N-acetyltransferase (*pat)* gene
Probe 2	5'-FAM-CTTCCAGGGCCTGGAGTCGTAC-TAMRA-3'
Target element	pGEM -specific spike probe
Amplicon length	68 bp (pat) and 108 bp (spike)

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L
Probe	0,20 µmol/L
Template DNA	500 ng
Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			40

Quantitative PCR method for detection of synthetic cryIA(b) gene

1. GENERAL INFORMATION

Target genetic element	Synthetic *cryIA(b)* gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/ELE/003

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Maize CRM flour
Materials used for calibration/controls	CRM IRMM-411 (JRC-IRMM)
Tested GM events	
Event Name	Bt 176
Unique Identifier	SYN-EV176-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

Each participant (n=17) received six blind samples consisting of four certified reference materials: 4 CRM IRMM-411 powders containing between 0.1% and 2% maize Event BT 176 (w/w), a maize/soya flour blind sample (50% GTS 40-3-2, 49% non-transgenic maize, and 1% maize Event BT 176) and a dried powder produced from heat sterilized kernels containing 2% maize Event BT 176. DNA was extracted twice and analyzed in triplicates. The standard curve method is used for DNA quantification. Separate calibration curves with each primer/probe system were generated within the same analytical amplification run.

Method Performance

LOD Relative	not reported	**LOD Absolute**	5 HGE
LOQ Relative	not reported	**LOQ Absolute**	50 HGE

Values determined in the collaborative trial

Test Level (%)	0.10%	0.50%	1.0%	2.0%
Mean Value (%)	0.13%	0.67%	1.5%	2.3%
RSD_r (%)	22%	19%	19%	20%
RSD_R (%)	36%	39%	37%	41%
Bias %	not reported	not reported	not reported	not reported

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The LOD and LOQ values were not assessed in this collaborative trial.

3. REFERENCES

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CCCATCGACATCAGCCTGAGC-3'
Target element	*cryIA(b)*
Primer Reverse	5'-CAGGAAGGCGTCCCACTGGC-3'
Target element	*cryIA(b)*
Amplicon length	129 bp
Probe	5'-FAM-ATGTCCACCAGGCCCAGCACG-TAMRA-3'
Target element	synthetic *cryIA(b)* gene

Taxon-target(s)

Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmgA*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmgA*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'
Target element	high-mobility-group A *(hmgA)* gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
Uracil-N-glycosylase (UNG)	0,5 U	Uracil-N-glycosylase (UNG)	0,5 U
AmpliTaq Gold® DNA Polymerase	1,25 U	AmpliTaq Gold® DNA Polymerase	1,25 U
TaqMan buffer A (with ROX™)	1x	TaqMan buffer A (with ROX™)	1x
$MgCl_2$	4,5 mmol/L	$MgCl_2$	4,5 mmol/L
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
Primer Fw	0,30 µmol/L	Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,30 µmol/L
Probe	0,16 µmol/L	Probe	0,16 µmol/L
Template DNA	2,3-150 ng	Template DNA	2,3-150 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45

Quantitative PCR method for detection of maize alcoholdeydrogenase 1 gene

1. GENERAL INFORMATION

Target element	alcohol dehydrogenase1 (*adh1*) gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/TAX/001

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Samples of maize DNA extracted from maize leaf material

Collaborative Trial Description

The method has been tested in a collaborative trial using six unknown samples. To establish a calibration curve, the participants received six samples of maize DNA extracted from leaf material and containing a known number of haploid maize genomes (absolute copy number expressed as haploid genome equivalents. The copy number of the calibration samples was calculated by dividing the sample DNA mass by the published average 1C value for maize genomes (2.725 pg). The expected copy numbers of the blind samples were determined with the procedure.

Method Performance

LOD Relative	not reported	**LOD Absolute**	10
LOQ Relative	not reported	**LOQ Absolute**	100

Values determined in the collaborative trial

Test Level (HGE)	**7339**	**18349**	**36697**	**55046**	**91743**	**14678**
Mean Value (HGE)	9985	23885	46918	75161	100541	12208
RSD_r (%)	13%	6.1%	12%	6%	11%	12%
RSD_R (%)	20%	8.7%	13%	9.1%	15%	15%
Bias %	36%	30%	28%	37%	9.6%	-17%

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R^2	not reported	not reported

Comment
The LOD and LOQ values were not assessed in the collaborative trial. According to the method developer the absolute LOD and LOQ were respectively 10 and 100 copies of the target sequence. The specificity of the method was previously tested against a wide range of non-target taxa and 20 different maize lines representing a geographical and phylogenetic wide sample. No-cross reactivity was observed with the non-target taxa (except with teosinte *Zea mays* subsp. *dilpoperennis*, the wild ancestor of cultivated maize).

3. REFERENCES

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	*adh1*
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	*adh1*
Amplicon length	135 bp
Probe	5'-FAM-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'
Target element	alcohol dehydrogenase1 (*adh1*) gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L
Probe	0,20 µmol/L
Template DNA	maximum 250
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			50

Quantitative PCR method for detection of tomato LAT52 gene

1. GENERAL INFORMATION

Target genetic element	*LAT52* gene
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT/TAX/002

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Jiao Tong University (GMDL-SJTU)
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from four tomato varieties, namely R144, Zhongsu5, Zaofeng and Lichum
Tested GM events	
Event Names	Not applicable
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

2.1 Collaborative Trial Description

Each participant received 12 genomic DNA from four different tomato varieties such as Zhongsu5, R144, Zaofeng, and Linchum (coded A, B, C, and D respectively) and serially diluted them to 50, 5, 0.5, 0.05, and 0.01 ng for PCR reaction to construct four standard curves. Eight blind tomato samples from these four cultivars were then quantified using the LAT52 real-time PCR assay. In addition participants received one positive DNA target control and one negative DNA control consisting of a salmon sperm DNA solution.

2.2 Method Performance

LOD Relative	not reported	**LOD Absolute**	≤0.01 ng
LOQ Relative	not reported	**LOQ Absolute**	≤0.01 ng

Values determined in the collaborative trial

Mean Value ng	0.45	0.48	0.49	0.49	0.046	0.047	0.052	0.049
RSD_r (%)	19%	16%	15%	19%	18%	23%	13%	13%
RSD_R (%)	27%	25%	22%	27%	34%	35%	32%	35%
Bias (%)	11%	3.9%	2.5%	2.3%	7.3%	7.1%	3%	1.9%

	Taxon Target
Mean Slope	not reported
Mean PCR Efficiency %	96
Mean R^2	1.00

Comment
The validation metrics and descriptive statistics were calculated from the data of three tests per level performed in three replicates.

3. REFERENCES

L. Yang, H Zhang,J. Guo, L. Pan, and D. Zhang (2008) "International Collaborative Study of the Endogenous Reference Gene LAT52 Used for Qualitative and Quantitative Analyses of Genetically Modified Tomato' J. Agric. Food Chem., Vol 56, p. 3438–3443

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-AGACCACGAGAACGATATTTGC-3'
Target element	*LAT52*
Primer Reverse	5'-TTCTTGCCTTTTCATATCCAGACA-3'
Target element	*LAT52*
Amplicon length	92 bp
Probe	5'-HEX-CTCTTTGCAGTCCTCCCTTGGGCT-BHQ-3'
Taxon Target element	*LAT52* gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
PCR Buffer	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	0,20 µmol/L each
Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L
Probe	0,20 µmol/L
HotStarTaq® DNA Polymerase	1,0 U
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	900”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	45”	
Denaturing, Annealing & Extension			40

Chapter 2:
Qualitative GMO detection PCR methods

Qualitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/001

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Tomato pulp
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.

Tested GM events

Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

Collaborative Trial Description

In this trial, participants received 10 samples of tomato pulp derived from the non-transgenic or the GM Tomato Nema 282F. Additionally one positive and one negative control were provided. The quality of the isolated DNA was tested using the endogenous polygalacturonase (*PG*) gene as a positive control. For detection of the genetic modification, five samples were tested with the primer pair 35S-1/35S-2, specific for the CaMV P-35S promoter. All PCR products were subsequently characterized by restriction analysis.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value has not been determined for this collaborative trial.

3. REFERENCES

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCTCCTACAAATGCCATCA-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-GATAGTGGGATTGTGCGTCA-3'
Target element	*CaMV P-35S*
Amplicon length	195 bp
Target element	CaMV 35S promoter

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp (endo) & 180 bp (insert)
Target element	polygalacturonase *(PG)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
Double-distilled sterile water	#	Double-distilled sterile water	#
AmpliTaq Gold® DNA Polymerase	2,0 U	AmpliTaq Gold® DNA Polymerase	2,0 U
PCR Buffer 10x (with $MgCl_2$)	1x	PCR Buffer 10x (with $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	50 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	50 µmol/L each
Primer Fw	0,40 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,40 µmol/L
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600”	1	94°C	600”	1
Denaturation	95°C	20”		94°C	30”	
Annealing	54°C	40”		60°C	60”	
Extension	72°C	40”		72°C	60”	
Denaturing, Annealing & Extension			35			35
Final Extension	72°C	180”	1	72°C	360”	1

Qualitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Biscuits (soybean)
Materials used for calibration/controls	In house produced processed food controls
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received a detailed method description for DNA extraction using either a CTAB method or a commercially available kit. PCR conditions had to be optimized for their local specific equipment. The method has been evaluated for the detection of genetically modified organisms in biscuits containing each 0%, 2%, and 10% of Roundup-Ready event GTS 40-3-2 soybeans. Each participant received control samples and unknown independent duplicates of GMO samples of which some contained 0% GMOs samples and others contained various percentages of the transgenic event. The participants were requested to analyse each sample once and to specify whether it was considered GMO positive or GMO negative using method for detecting the CaMV P-35S promoter.

Method Performance

LOD Relative	≤2%	**LOD Absolute**	50 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	6.7%
False negative (%)	1.5%

Test Level (%)	0%	2%	10%
Specificity %	93%	-	-
Sensitivity %	-	100%	97%

Comment
The samples were prepared by the collaborative trial coordinator following procedures that resemble as closely as possible the different processing conditions applied by the food industry. The absolute and relative LOD have not been determined for this method.

3. REFERENCES

M. Lipp, A. Bluth, F. Eyquem, L. Kruse, H. Schimmel, G. Van den Eede and E. Anklam (2001) Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs Eur. Food Res. Technol. 212: 497-504

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CCACGTCTTCAAAGCAAGTGG-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-TCCTCTCCAAATGAAATGAACTTCC-3'
Target element	*CaMV P-35S*
Amplicon length	123 bp
Target element	CaMV 35S promoter

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
Water	#
AmpliTaq Gold® DNA Polymerase	0,8 IU
PCR Buffer 10x (with 15 mmol/L $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	640 µmol/L
Primer Fw	0,60 µmol/L
Primer Rev	0,60 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	25"	
Annealing	62°C	30"	
Extension	72°C	45"	
Denaturing, Annealing & Extension			50
Final Extension	72°C	420"	1

Qualitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/005

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Soybean grain
Materials used for calibration/controls	CRM IRMM-410 (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received five encoded samples of transgenic (100% soybean event GTS 40-3-2 and non-transgenic soybean grain. The laboratories had to extract DNA and analyse the corresponding samples with primers specific for the CaMV 35S promoter. The quality of the isolated DNA was tested using a specific sequence from the lectin gene (*Le1*). The identity of the amplicons was confirmed by restriction enzyme digestion.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	-	**LOQ Absolute**	-

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	**0%**	**100%**
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The reported LOD value was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCTCCTACAAATGCCATCA-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-GATAGTGGGATTGTGCGTCA-3'
Target element	*CaMV P-35S*
Amplicon length	195 bp
Target element	CaMV 35S promoter

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCATCC-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTTGTG-3'
Target element	*Le1*
Amplicon length	118 bp
Target element	lectin *(Le1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1 x	PCR Buffer 10x (without $MgCl_2$)	1 x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
AmpliTaq Gold® DNA Polymerase	0,5 IU	AmpliTaq Gold® DNA Polymerase	0,5 IU
Sterile water	#	Sterile water	#
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	94°C	20”		95°C	30”	
Annealing	54°C	40”		60°C	30”	
Extension	72°C	60”		72°C	60”	
Denaturing, Annealing & Extension			40			35
Final Extension	72°C	180”	1	72°C	180”	1

Qualitative duplex PCR method for detection of Cauliflower Mosaic Virus 35S promoter and nopaline synthase terminator (partim CaMV P-35S)

1. GENERAL INFORMATION

Target genetic elements	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Duplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/ELE/012

2. VALIDATION DATA

Collaborative trial coordinator	Chemisches und Veterinaruntersuchungsamt Freiburg
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	CRM BF412F (JRC-IRMM)
Tested GM events	
Event Names	Bt11; MON810; GA21
Unique Identifier	SYN-BT011-1; MON-00810-6; MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

The inter-laboratory study was conducted to evaluate a duplex real-time PCR screening method for the simultaneous detection and semi-quantitative estimation of CaMV 35S promoter and T-nos sequences in transgenic maize reference samples. Participants received nine different maize DNA mixtures containing the transformation events Bt11, MON 810 and/or GA21 at different concentration levels (see endnote). In addition each laboratory received one negative GM maize DNA sample, DNA calibration standards extracted from Bt11 reference material for the quantification of the CaMV P-35S and T-nos sequence and reagents. Each DNA sample had to be analyzed in five replicas.

Method Performance

LOD Relative	≤0.02%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial (partim CaMV P-35S)

False positive (%)	18%
False negative (%)	not reported

Test Level	1	2	3	4	5	6	7	8	9
Sensitivity (%)	100%	100%	100%	100%	100%	-	-	100%	100%
Mean Value	13	56	470	1170	29			43	1192
RSD_r (%)	38%	27%	15%	10%	32%	-	-	42%	13%
RSD_R (%)	-	-	-	-	-	-	-	-	-

	GMO Target
Mean Slope	not reported
Mean PCR Efficiency %	93%
Mean R^2	not reported

Comment
The relative LOD was not determined in this collaborative trial.

3. REFERENCES

H.-U. Waiblinger, B. Ernst, A. Anderson, and K. Pietsch "Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modiWed organisms in food products" Eur Food Res Technol (DOI 10.1007/s00217-007-0748-z)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCCTCTGCCGACAGTGGT-3' 5'-CATGTAATGCATGACGTTATTTATG-3'
Target element	*CaMV P-35S* *T-nos*
Primer Reverse	5'-AAGACGTGGTTGGAACGTCTTC-3' 5'-TTGTTTTCTATCGCGTATTAAATGT-3'
Target element	*CaMV P-35S* *T-nos*
Amplicon length	82 bp 84 bp
Probe	5'-FAM-CAAAGATGGACCCCCACCCACG-BHQ1-3' 5'-YY-ATGGGTTTTTATGATTAGAGTCCCGCAA-BHQ1-3'
GMO Target element	CaMV 35S promoter and nopaline synthase terminator

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer 35S-F	0,10 µmol/L
Primer 35S-R	0,10 µmol/L
Probe 35S-TMP FAM	0,10 µmol/L
Primer 180-F	1,0 µmol/L
Primer 180-R	1,0 µmol/L
Probe TM-180 YY	0,20 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Endnote on the composition of the respective tested combinations:

0.02% Bt11 (CAMV P-35S+; T-nos+)(Level 1), 0.1% Bt11 (CAMV P-35S+; T-nos+) (Level 2), 1.0% Bt11 (CAMV P-35S+; T-nos+) (Level 3), 0.05% MON810 (CAMV P-35S+; T-nos-) (Level 4), 2.5% MON810 (CAMV P-35S+; T-nos-) (Level 5), 0.05% GA21 (CAMV P-35S-;T-nos+) (Level 6), 2.5% GA21 (CAMV P-35S-; T-nos+) (Level 7), 0.05% MON810 + 2.5% GA21 (CAMV P-35S+; T-nos+) (Level 8) and 2.5% MON810 + 0.05% GA21 (CAMV P-35S+; T-nos+) (Level 9)

Qualitative PCR method for detection of chloroplast tRNA-Leu intron

1. GENERAL INFORMATION

Target genetic element	Chloroplast tRNA-Leu (trnL) intron
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/008

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	potato tissue
Materials used for calibration/controls	potato materials
Tested GM events	
Event Name	B33-INV
Unique Identifier	not applicable
Crop Name	*Solanum tuberosum L.*

Collaborative Trial Description

All laboratories received encoded samples of two non-genetically modified potato cultivars and of genetically modified potato type (B33-INV). A method for detecting a chloroplast tRNA gene which is generally present as a multicopy DNA sequence in plant chloroplasts was used to demonstrate the presence of potato tissue in a sample. The identity of the amplicon could be confirmed by Southern blotting with a specific labeled DNA probe or by sequencing.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value has not been determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs Methods of analysis for the detection of genetically modified organisms and derived products. Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (1997). Food Analysis, L 24.01-1. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-CGAAATCGGTAGACGCTACG-3'
Target element	*trnL*
Primer Reverse	5'-GGGGATAGAGGGACTTGAAC-3'
Target element	*trnL*
Amplicon length	571 bp
Target element	chloroplast *trnL* intron

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L
Primer Fw	0,80 µmol/L
Primer Rev	0,80 µmol/L
AmpliTaq DNA polymerase	0,5 IU
Sterile water	#
Template DNA	10-50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	94°C	240”	1
Denaturation	95°C	30”	
Annealing	55°C	30”	
Extension	72°C	120”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	300”	1

Qualitative PCR method for detection of Figwort Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Figwort Mosaic Virus 35S promoter (P-FMV)
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/010

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Entry-Exit Inspection & Quarantine Bureau
Test material applied in collaborative trial	Oilseed rape meal
Materials used for calibration/controls	Meal derived from dried grain samples of oilseed rape event GT73 (Monsanto)
Tested GM events	
Event Name	GT73 (RT73)
Unique Identifier	MON-00073-7
Crop Name	*Brassica napus* L.

Collaborative Trial Description

Each laboratory received 20 encoded dried meal samples, including 10 samples for the detection of the oilseed rape high-mobility-group A (*hmgA*) endogene and 10 samples for the detection of the FMV 35S promoter. The first series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) non-GM oilseed rape in a rice background. The second series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) GT73 oilseed rape in non-GM oilseed rape samples. The participants were asked to extract DNA from the samples according to the protocol provided. The amplified PCR products were analyzed by electrophoresis. The detection of a DNA fragment with the same size as the positive control indicated that the sample was positive; otherwise the result was considered negative.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	75 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	not reported
False negative (%)	23%

Test Level (%)	**0.01%**	**0.05%**	**0.1%**	**1%**	**5%**
Sensitivity (%)	21%	71%	96%	100%	100%

Comment
The LOD was not determined in this collaborative trial.

3. REFERENCES

L. Pan, S. Zhang, L. Yang, H. Broll, F. Tian, and D. Zhang (2007) "Interlaboratory Trial Validation of an Event-Specific Qualitative Polymerase Chain Reaction-Based Detection Method for Genetically Modified RT73 Rapeseed" Journal of AOAC International, Vol. 90, No. 6, p. 1639-1646

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AAGCCTCAACAAGGTCAG-3'
Target element	*P-FMV*
Primer Reverse	5'-CTGCTCGATGTTGACAAG-3'
Target element	*P-FMV*
Amplicon length	196 bp
GMO Target element	FMV 35S promoter

Taxon-target(s)

Primer Forward	5'-GGTCGTCCTCCTAAGGCGAAAG-3'
Target element	*hmgA*
Primer Reverse	5'-GCAACCAACAGGCACCATC-3'
Target element	*hmgA*
Amplicon length	219 bp
Taxon Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
Taq DNA Polymerase buffer 10x	1x	Taq DNA Polymerase buffer 10x	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
Taq DNA Polymerase	1,0 U	Taq DNA Polymerase	1,0 U
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	94°C	180”	1	94°C	180”	1
Denaturation	94°C	30”		94°C	30”	
Annealing	54°C	30”		59°C	30”	
Extension	72°C	40”		72°C	40”	
Denaturing, Annealing & Extension			40			40
Final Extension	72°C	180”	1	72°C	180”	1

Qualitative PCR method for detection of neomycin phosphotransferase II gene

1. GENERAL INFORMATION

Target genetic element	Neomycin phosphotransferase II (*nptII*) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/002

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Tomato pulp
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.
Tested GM events	
Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

Collaborative Trial Description

In this trial, participants received 10 samples of tomato pulp derived from the non-transgenic or the genetically modified Tomato Nema 282F. Additionally one positive and one negative control were provided. The quality of the isolated DNA was tested using the endogenous polygalacturonase (*PG*) gene as a positive control. For detection of the neomycin phosphotransferase II (*nptII*) gene in the genetic modification, five samples were tested with the primer pair TN5-1/TN5-2. The PCR products were subsequently characterized by restriction analysis.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value has not been determined for this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CTCACCTTGCTCCTGCCGAGA-3'
Target element	*nptII*
Primer Reverse	5'-CGCCTTGAGCCTGGCGAACAG-3'
Target element	*nptII*
Amplicon length	215 bp
Target element	neomycin phosphotransferase II (*nptII)* gene

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp (endo)+180 bp (insert)
Target element	polygalacturonase *(PG)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
Water	#	Water	#
PCR Buffer 10x (with $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L	$MgCl_2$	1,5 mmol/L
Primer Fw	0,40 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Rev	0,40 µmol/L	Primer Fw	0,40 µmol/L
AmpliTaq Gold® DNA Polymerase	2,0 IU	Primer Rev	0,40 µmol/L
Template DNA	5 µL	AmpliTaq Gold® DNA Polymerase	1,0 IU
Final Volume	25 µL	Template DNA	10-50 ng
Final Volume	25 µL		

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	Temperature	Time	No Cycles	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	25”		94°C	30”	
Annealing	60°C	30”		60°C	60”	
Extension	72°C	45”		72°C	60”	
Denaturing, Annealing & Extension			35			35
Final Extension	72°C	420”	1	72°C	360”	1

Qualitative PCR method for detection of neomycin phosphotransferase II gene

1. GENERAL INFORMATION

Target genetic element	Neomycin phosphotransferase II (*nptII*) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/003

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Tomato pulp
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.
Tested GM events	
Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

Collaborative Trial Description

In this trial, participants received 10 samples of tomato pulp derived from the non-transgenic or the genetically modified Tomato Nema 282F. Additionally one positive and one negative control were provided. The quality of the isolated DNA was tested using the endogenous polygalacturonase (*PG*) gene as a positive control. For detection of the neomycin phosphotransferase II (*nptII*) gene in the genetic modification, all samples were tested with the primer pair TN5-1/TN5-2. The PCR products were subsequently characterized by restriction analysis.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value has not been determined for this collaborative trial.

3. REFERENCES

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GGATCTCCTGTCATCT-3'
Target element	*nptII*
Primer Reverse	5'-GATCATCCTGATCGAC-3'
Target element	*nptII*
Amplicon length	173 bp
Target element	neomycin phosphotransferase II (*nptII*) gene

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp & 180 bp
Target element	polygalacturonase *(PG)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
Double-distilled sterile water	#	Water	#
AmpliTaq Gold® DNA Polymerase	2,0 U	$MgCl_2$	1,5 mmol/L
PCR Buffer 10x (with MgCl2)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	50 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Fw	0,40 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,40 µmol/L
Template DNA	10-50 ng	AmpliTaq Gold® DNA Polymerase	1,0 IU
Final Volume	50 µL	Template DNA	10-50 ng
Final Volume	25 µL		

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	Temperature	Time	No Cycles	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	25”		94°C	30”	
Annealing	60°C	30”		60°C	60”	
Extension	72°C	45”		72°C	60”	
Denaturing, Annealing & Extension			35			35
Final Extension	72°C	420”	1	72°C	360”	1

Qualitative PCR method for detection of nopaline synthase terminator

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/006

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	soybeans
Materials used for calibration/controls	CRM IRMM-410 (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received five encoded samples of transgenic (100% soybean event GTS 40-3-2) and non-transgenic soybean grain. The laboratories had to extract DNA and analyse the corresponding samples with primers specific for the T-nos terminator. The quality of the isolated DNA was tested using a specific sequence from the lectin gene (*Le1*). The identity of the amplicons was confirmed by restriction enzyme digestion.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value was not determined in this collaborative trial.

3. REFERENCES

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). FoodAnalysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GAATCCTGTTGCCGGTCTTG-3'
Target element	*T-nos*
Primer Reverse	5'-TTATCCTAGTTTGCGCGCTA-3'
Target element	*T-nos*
Amplicon length	180 bp
Target element	nopaline synthase terminator

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCATCC-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTTGTG-3'
Target element	*Le1*
Amplicon length	118 bp
Target element	lectin *(Le1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x	1x	PCR Buffer 10x	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
AmpliTaq Gold® DNA polymerase	0,5 -1 U	AmpliTaq DNA polymerase	0,5-1 U
Double-distilled sterile water	#	Double-distilled sterile water	#
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	20”		95°C	30”	
Annealing	54°C	40”		60°C	30”	
Extension	72°C	40”		72°C	60”	
Denaturing, Annealing & Extension			35			35
Final Extension	72°C	180”	1	72°C	180”	1

Qualitative PCR method for detection of nopaline synthase terminator

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Single
Detection Chemistry	Agarose gelelectrophoresis
Compendium Reference	SC/ELE/007

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	raw tomato
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.
Tested GM events	
Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

Collaborative Trial Description

The participants received 10 samples of tomato pulp derived from the unmodified control organism or the genetically modified Zeneca tomato. Additionally one positive and one negative control were provided. The DNA extracted from the samples had to be tested with a control primer pair that amplifies a sequence of 384 bp from the endogenous PG gene. With this primer pair an additional fragment of 180 bp is obtained in the samples of the GM Zeneca tomato only. The respective samples had to be amplified with primer pair nos-1/nos-3 (specific for the nopaline synthase (T-nos) terminator). PCR products were subsequently characterized by restriction analysis.

Method Performance

LOD Relative	not reported	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	10%
Specificity %	100%	-
Sensitivity %	-	100%

3. REFERENCES

EU-Project SMT4-CT96-2072. Developments of Methods to Identify Foods Produced by Means of Genetic Engineering Techniques (DMIF-GEN). Final Report

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GAATCCTGTTGCCGGTCTTG-3'
Target element	*T-nos*
Primer Reverse	5'-TTATCCTAGTTTGCGCGCTA-3'
Target element	*T-nos*
Amplicon length	180 bp
GMO Target element	nopaline synthase terminator

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp (+180 bp
Taxon Target element	polygalacturonase (*PG*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
Water	#	Water	#
AmpliTaq Gold® DNA Polymerase	2 U	AmpliTaq Gold® DNA Polymerase	1 IU
PCR Buffer 10x with $MgCl_2$)	1x	PCR Buffer 10x with $MgCl_2$)	1x
$MgCl_2$	#	$MgCl_2$	1.5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	50 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Fw	0,40 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,40 µmol/L
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	50 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	20”		94°C	30”	
Annealing	54°C	40”		60°C	60”	
Extension	72°C	40”		72°C	60”	
Denaturing, Annealing & Extension			35			35
Final Extension	72°C	180”	1	72°C	360”	1

Qualitative PCR method for detection of nopaline synthase terminator

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/ELE/009

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Biscuits (soybean+maize)
Materials used for calibration/controls	In house produced processed food controls
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received a detailed method description for DNA extraction using either a CTAB method or a commercially available kit. PCR conditions had to be optimized for their local specific equipment. The method has been evaluated for the detection of genetically modified organisms in biscuits containing each 0%, 2%, and 10% of event GTS 40-3-2 soybeans. Each participant received control samples and unknown independent duplicates of GMO samples of which some contained 0% GMOs samples and others contained various percentages of the transgenic event. The participants were requested to analyse each sample once and to specify whether it was considered GMO positive or GMO negative using method for detecting the T-nos terminator.

Method Performance

LOD Relative	≤2%	**LOD Absolute**	50 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	1.7%

Test Level (%)	0%	2%	10%
Specificity %	100%	-	-
Sensitivity %	-	97%	100%

Comment
The samples were prepared by the collaborative trial coordinator following procedures that resemble as closely as possible the different processing conditions applied by the food industry. The absolute and relative LOD have not been determined for this method.

3. REFERENCES

M. Lipp, A. Bluth, F. Eyquem, L. Kruse, H. Schimmel, G. Van den Eede and E. Anklam (2001) Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs Eur. Food Res. Technol. 212: 497-504

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCATGACGTTATTTATGAGATGGG-3'
Target element	*T-nos*
Primer Reverse	5'-GACACCGCGCGCGATAATTTATCC-3'
Target element	*T-nos*
Amplicon length	118 bp
Target element	nopaline synthase terminator

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
Water	#
AmpliTaq Gold® DNA Polymerase	0,8 IU
PCR Buffer 10x (with 15 mmol/L $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	640 µmol/L
Primer Fw	0,60 µmol/L
Primer Rev	0,60 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	25”	
Annealing	62°C	30”	
Extension	72°C	45”	
Denaturing, Annealing & Extension			50
Final Extension	72°C	420”	1

Qualitative PCR method for detection of nopaline synthase terminator

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/ELE/011

2. VALIDATION DATA

Collaborative trial coordinator	Federal Office for Consumer Protection and Food Safety (BVL)
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	CRM BF-415b and BF-415c (JRC-IRMM)
Tested GM events	
Event Name	NK 603
Unique Identifier	MON-00603-6
Crop Name	*Zea mays* L.

Collaborative Trial Description

All 12 laboratories received 18 blind DNA samples of which 12 were prepared from GMO CRM (0.1% or 0.5% maize event NK 603) and six from non-GM maize flour. In addition, participants received a positive DNA target control consisting of a solution of 0.5% maize event NK 603 DNA and solutions of primers, probe and a commercial reagent kit. The participants were requested to submit the cycle threshold (Ct) values obtained in the T-nos real-time PCR with each sample and to report whether the sample was considered positive or negative.

Method Performance

LOD Relative	not reported	**LOD Absolute**	8 target copies
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	2.2%
False negative (%)	0%

Test Level (%)	0%	0.1%	0.5%
Specificity %	98%	-	-
Sensitivity %	-	100%	100%

Comment
The laboratories were requested to analyze the unknown samples with a maize taxon-specific PCR method of their choice in order to verify the presence and quality of the sample DNA. This step was not included into the evaluation of the collaborative trial.

3. REFERENCES

R. Reiting, H. Brol, H.-U. Waiblinger, and L. Grohmann (2007) "Collaborative Study of a T-NOS Real-Time PCR Method for Screening of Genetically Modified Organisms in Food Products" Journal für Verbraucherschutz und Lebensmittelsicherheit, Vol 2, p.116–121 (DOI 10.1007/s00003-007-0189-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CATGTAATGCATGACGTTATTTATG-3'
Target element	*T-nos*
Primer Reverse	5'-TTGTTTTCTATCGCGTATTAAATGT-3'
Target element	*T-nos*
Amplicon length	84 bp
Probe	5'-FAM-ATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA-3'
GMO Target element	nopaline synthase terminator

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
QuantiTect® QuantiTect Probe PCR Master Mix (Qiagen)	1x
Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L
Probe	0,10 µmol/L
Template DNA	100 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	900”	1
Denaturation	94°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Qualitative duplex PCR method for detection of Cauliflower Mosaic Virus 35S promoter and nopaline synthase terminator (partim T-nos)

1. GENERAL INFORMATION

Target genetic elements	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Duplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/ELE/013

2. VALIDATION DATA

Collaborative trial coordinator	Chemisches und Veterinaruntersuchungsamt Freiburg
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	CRM BF412F (JRC-IRMM)
Tested GM events	
Event Names	Bt11; MON 810; GA21
Unique Identifier	SYN-BT011-1; MON-00810-6; MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

The inter-laboratory study was conducted to evaluate a duplex real-time PCR screening method for the simultaneous detection and semi-quantitative estimation of CaMV 35S promoter and T-nos sequences in transgenic maize reference samples. Participants received nine different maize DNA mixtures containing the transformation events Bt11, MON 810 and/or GA21 at different concentration levels (see endnote). In addition each laboratory received one negative GM maize DNA sample, DNA calibration standards extracted from Bt11 reference material for the quantification of the CaMV P-35S and T-nos sequence and reagents. Each DNA sample had to be analyzed in five replicas.

Method Performance

LOD Relative	≤0.02%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial (partim T-nos)

False positive (%)	3.3%
False negative (%)	not reported

Test Level	1	2	3	4	5	6	7	8	9
Sensitivity (%)	98%	100%	100%		-	100%	100%	100%	100%
Mean Value	5.7	37	404	-	-	4500	81	4650	69
RSD_r (%)	61%	35%	25%	-	-	38%	28%	40%	40%
RSD_R (%)	-	-	-	-	-	-	-	-	-

	GMO Target
Mean Slope	not reported
Mean PCR Efficiency (%)	102%
Mean R^2	not reported

Comment
The relative LOD was not determined in this collaborative trial.

3. REFERENCES

H.-U. Waiblinger, B. Ernst, A. Anderson, and K. Pietsch "Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modiWed organisms in food products" Eur Food Res Technol (DOI 10.1007/s00217-007-0748-z)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCCTCTGCCGACAGTGGT-3' 5'-CATGTAATGCATGACGTTATTTATG-3'
Target element	*CaMV P-35S* *T-nos*
Primer Reverse	5'-AAGACGTGGTTGGAACGTCTTC-3' 5'-TTGTTTTCTATCGCGTATTAAATGT-3'
Target element	*CaMV P-35S* *T-nos*
Amplicon length	82 bp 84 bp
Probe	5'-FAM-CAAAGATGGACCCCCACCCACG-BHQ1-3' 5'-YY-ATGGGTTTTTATGATTAGAGTCCCGCAA-BHQ1-3'
GMO Target element	CaMV 35S promoter and nopaline synthase terminator

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer 35S-F	0,10 µmol/L
Primer 35S-R	0,10 µmol/L
Probe 35S-TMP FAM	0,10 µmol/L
Primer 180-F	1,0 µmol/L
Primer 180-R	1,0 µmol/L
Probe TM-180 YY	0,20 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Endnote on the composition of the respective tested combinations:

0.02% Bt11 (CAMV P-35S+; T-nos+)(Level 1), 0.1% Bt11 (CAMV P-35S+; T-nos+) (Level 2), 1.0% Bt11 (CAMV P-35S+; T-nos+) (Level 3), 0.05% MON810 (CAMV P-35S+; T-nos-) (Level 4), 2.5% MON810 (CAMV P-35S+; T-nos-) (Level 5), 0.05% GA21 (CAMV P-35S-;T-nos+) (Level 6), 2.5% GA21 (CAMV P-35S-; T-nos+) (Level 7), 0.05% MON810 + 2.5% GA21 (CAMV P-35S+; T-nos+) (Level 8) and 2.5% MON810 + 0.05% GA21 (CAMV P-35S+; T-nos+) (Level 9)

Qualitative PCR method for detection of phosphinothricin N-acetyltransferase gene

1. GENERAL INFORMATION

Target genetic element	Phosphinothricin N-acetyltransferase (*bar*) gene from bacterium *Streptomyces hygroscopicus*
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/ELE/014

2. VALIDATION DATA

Collaborative trial coordinator	Federal Office for Consumer Protection and Food Safety (BVL)
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	Genomic DNA (Bayer CropScience)
Tested GM events	
Event Name	MS8; LLRice62
Unique Identifier	ACS-BN005-8; ACS-OS002-5
Crop Name	*Brassica napus;* Oryza sativa L.

Collaborative Trial Description

A set of 18 encoded samples was sent to all laboratories, containing 12 GM-positive (analyte levels at 0.02%, and 0.1% GM) and 6 GM-negative DNA samples. To assess the limit of detection, precision and accuracy of the method, laboratories had to analyze the encoded genomic DNA samples of the oilseed rape event MS8 and rice event LLRice62. In addition, standard DNAs were provided to the laboratories to generate calibration curves for copy number quantification of the *bar* target sequences.

Method Performance

LOD Relative	≤0.02%	**LOD Absolute**	not reported
LOQ Relative	≤0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	**0%**	**0.02%**	**0.1%**
Specificity %	100%	-	-

Sensitivity %	-	100%	100%
Mean Value (%)	-	0.03%	0.11%
RSD_r (%)	-	not reported	not reported
RSD_R (%)	-	31%	17%
Bias (%)	-	not reported	not reported

	GMO Target
Mean Slope	-3.09
Mean PCR Efficiency %	111
Mean R^2	0.98

Comment
The blind samples for the *bar* detection were prepared by a laboratory not involved in this collaborative trial.

3. REFERENCES

L. Grohmann, C. Brünen-Nieweler, A. Nemeth, and H.-U. Waiblinger (2009) "Collaborative Trial Validation Studies of Real-Time PCR-Based GMO Screening Methods for Detection of the bar Gene and the ctp2-cp4 epsps Construct" J. Agric. Food Chem. Vol 57, p. 8913–8920 (DOI:10.1021/jf901598r)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-ACAAGCACGGTCAACTTCC-3'
Target element	*bar*
Primer Reverse	5'-GAGGTCGTCCGTCCACTC-3'
Target element	*bar*
Amplicon length	60 bp
Probe	5'-FAM-TACCGAGCCGCAGGAACC-TAMRA-3'
Target element	*bar* gene

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
QuantiTect Multiplex PCR No-ROX™	1x
Primer Fw	0,34 µmol/L
Primer Rev	0,34 µmol/L
Probe	0,34 µmol/L
Template DNA	200 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	900"	1
Denaturation	95°C	10"	
Annealing & Extension	60°C	15"	
Denaturing, Annealing & Extension			45

Qualitative PCR method for detection of the junction between the chloroplast transit peptide 2 and the CP4 epsps gene

1. GENERAL INFORMATION

Target genetic element	Junction region between the chloroplast transit peptide 2 (CTP2) sequence from the *Arabidopsis thaliana epsps* gene and the CP4 *epsps* gene from *Agrobacterium tumefasciens* (CP4-EPSPS)
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/CON/008

2. VALIDATION DATA

Collaborative trial coordinator	Federal Office for Consumer Protection and Food Safety (BVL)
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	GT73 oilseed rape flour (AOCS 0304-B)
Tested GM events	
Event Name	GT73 (RT73) ; NK603
Unique Identifier	MON-00073-7 ; MON-00603-6
Crop Name	*Brassica napus* L.; *Zea mays* L.

2.1 Collaborative Trial Description

A set of 18 encoded samples was sent to all laboratories, containing 12 GM-positive (analyte levels at 0.02%, and 0.1% GM) and 6 GM-negative DNA samples. To assess the limit of detection, precision and accuracy of the method, laboratories had to analyze the coded genomic DNA samples of the oilseed rape event GT73. In addition, standard DNAs were provided to the laboratories to generate calibration curves for copy number quantification of the *ctp2-CP4 epsps* target sequence.

2.2 Method Performance

LOD Relative	≤0.02%	**LOD Absolute**	not reported
LOQ Relative	≤0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	3%
False negative (%)	0%

Test Level (%)	0%	0.02%	0.1%
Specificity %	97%	-	-
Sensitivity %	-	100%	100%
Mean Value (%)	-	0.018%	0.072%
RSD_r (%)	-	not reported	not reported
RSD_R (%)	-	50%	32%
Bias (%)	-	not reported	not reported

	GMO Target
Mean Slope	-3.33
Mean PCR Efficiency (%)	103
Mean R^2	0.99

Comment
The blind samples for the *ctp2-cp4epsps* target detection were prepared by a laboratory not involved in this collaborative trial.

3. REFERENCES

L. Grohmann, C. Brünen-Nieweler, A. Nemeth, and H.-U. Waiblinger (2009) "Collaborative Trial Validation Studies of Real-Time PCR-Based GMO Screening Methods for Detection of the bar Gene and the ctp2-cp4epsps Construct" J. Agric. Food Chem. Vol 57, p. 8913–8920 (DOI:10.1021/jf901598r)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GGGATGACGTTAATTGGCTCTG-3'
Target element	*CTP2*
Primer Reverse	5'-GGCTGCTTGCACCGTGAAG-3'
Target element	*CP4-EPSPS*
Amplicon length	88 bp
Probe	5'-FAM-CACGCCGTGGAAACAGAAGACATGACC-TAMRA-3'
Target element	DNA sequence within the junction region

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,375 µmol/L
Primer Rev	0,375 µmol/L
Probe	0,15 µmol/L
Template DNA	65 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Qualitative PCR method for detection of maize event Bt11

1. GENERAL INFORMATION

Target genetic element	Junction region between the Intron 2 (IVS2) from the maize alcohol dehydrogenase 1 (*adh1*) gene and the phosphinothricin N-acetyltransferase gene (*pat*) from *Streptomyces viridochromogenes*
PCR Assay	Single
Detection Chemistry	Agarose Gelectrophoresis
Compendium Reference	SC/CON/003

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Maize kernels
Materials used for calibration/controls	CRM IRMM-412, (JRC-IRMM)
Tested GM events	
Event Name	Bt11
Unique Identifier	SYN-BT011-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

The laboratories tested 6 encoded samples consisting of maize event Bt11 maize and non-transgenic / event Bt176 maize mixtures. Of each sample, two independent DNA extractions were analyzed in duplicate. Quality of the isolated DNA was tested using the maize invertase gene as a positive control. Confirmation of the PCR products was carried out by restriction analysis or by Southern blot hybridization.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	4.7%
False negative (%)	9.4%

Test Level (%)	0%	100%
Specificity %	95%	-
Sensitivity %	-	91%

Comment
The reported LOD value was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (2001). Food Analysis, L15.05-01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CTGGGAGGCCAAGGTATCTAAT-3'
Target element	*IVS 2 adh1*
Primer Reverse	5'-GCTGCTGTAGCTGGCCTAATCT-3'
Target element	*pat*
Amplicon length	189 bp
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CCGCTGTATCACAAGGGCTGGTACC-3'
Target element	*ivr1*
Primer Reverse	5'-GGAGCCCGTGTAGAGCATGACGATC-3'
Target element	*ivr1*
Amplicon length	225 bp
Target element	invertase *(ivr1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	2 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Water	#	Water	#
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 IU	AmpliTaq Gold® DNA Polymerase	1,0 IU
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	720”	1	95°C	720”	1
Denaturation	95°C	30”		95°C	30”	
Annealing	64°C	30”		64°C	30”	
Extension	72°C	30”		72°C	60”	
Denaturing, Annealing & Extension			38			35
Final Extension	72°C	600”	1	72°C	600”	1

Qualitative PCR method for detection of maize event Bt 176

1. GENERAL INFORMATION

Target genetic element	Junction region between a calcium-dependent protein kinase promoter (P-CDPK) from maize and a synthetic *cryIA(b)* gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/CON/004

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	CRM IRMM-411 (JRC-IRMM)
Tested GM events	
Event Name	Bt176
Unique Identifier	SYN-EV176-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

All laboratories tested 6 encoded samples consisting of maize event Bt11 and non-transgenic/event Bt 176 maize mixtures. Of each sample, two independent DNA extractions were analyzed in duplicate. Quality of the isolated DNA was tested using the maize invertase (*ivr1*) gene as a positive control. Confirmation of the PCR products was carried out by restriction analysis or by Southern blot hybridization.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	not reported
False negative (%)	7.7%

Test Level (%)	**0.1%**
Sensitivity %	92%

Comment
The reported LOD value was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modifiedorganisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (2001). Food Analysis, L15.05-01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CTCTCGCCGTTCATGTCCGT-3'
Target element	*P-CDPK*
Primer Reverse	5'-GGTCAGGCTCAGGCTGATGT-3'
Target element	*cryIA(b)*
Amplicon length	211 bp
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CCGCTGTATCACAAGGGCTGGTACC-3'
Target element	*Ivr1*
Primer Reverse	5'-GGAGCCCGTGTAGAGCATGACGATC-3'
Target element	*Ivr1*
Amplicon length	225 bp
Target element	invertase *(ivr1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Water	#	Water	#
Primer Fw	0,25 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,25 µmol/L	Primer Rev	0,50 µmol/L
AmpliTaq Gold® DNA Polymerase	0,5 IU	AmpliTaq Gold® DNA Polymerase	1,0 IU
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	720”	1	95°C	720”	1
Denaturation	95°C	30”		95°C	30”	
Annealing	63°C	30”		64°C	30”	
Extension	72°C	30”		72°C	60”	
Denaturing, Annealing & Extension			38			35
Final Extension	72°C	360”	1	72°C	600”	1

Qualitative PCR method for detection of maize event T25

1. GENERAL INFORMATION

Target genetic element	Junction region between the phosphinothricin N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes* and the Cauliflower Mosaic Virus 35S terminator (CaMV T-35S)
PCR Assay	Single
Detection Chemistry	Agarose gel electrophoresis
Compendium Reference	SC/CON/005

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	Flour of maize event T25 grinded kernels
Tested GM events	
Event Name	T25
Unique Identifier	ACS-ZM003-2
Crop Name	*Zea mays* L.

Collaborative Trial Description

All laboratories received 5 encoded samples from flour (ground kernels) of maize event T25 (0.1%, 1%), maize event MON810 (0.1%, 1%) and non-transgenic maize. Of each sample, two independent DNA extractions had to be analyzed in duplicate. Quality of the isolated DNA was tested using the maize invertase (*ivr1*) gene as a positive control. Confirmation of the PCR products was carried out by restriction enzyme analysis.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	12%

Test Level (%)	0%	0.1%	1%
Specificity %	100%	-	-
Sensitivity %	-	83%	93%

Comment
All test materials (including the controls) were prepared by the collaborative trial coordinator. The reported LOD value was not reported in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (2001). Food Analysis, L15.05-01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-ATGGTGGATGGCATGATGTTG-3'
Target element	*pat*
Primer Reverse	5'-TGAGCGAAACCCTATAAGAACCC-3'
Target element	*CaMV T-35S*
Amplicon length	209 bp
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-CCGCTGTATCACAAGGGCTGGTACC-3'
Target element	*Ivr1*
Primer Reverse	5'-GGAGCCCGTGTAGAGCATGACGATC-3'
Target element	*Ivr1*
Amplicon length	225 bp
Target element	invertase (*ivr1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	2 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Water	#	Water	#
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 IU	AmpliTaq Gold® DNA Polymerase	1,0 IU
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	720”	1	95°C	720”	1
Denaturation	95°C	30”		95°C	30”	
Annealing	64°C	30”		64°C	30”	
Extension	72°C	30”		72°C	60”	
Denaturing, Annealing & Extension			40			35
Final Extension	72°C	600”		72°C	600”	1

Qualitative PCR method for detection of rice event Bt63

1. GENERAL INFORMATION

Target genetic element	Junction region between a cry1A(b)/cry1A(c) fusion gene and the DNA spacer sequences linking the fusion gene to the nopaline synthase terminator (T-nos)
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	SC/CON/007

2. VALIDATION DATA

Collaborative trial coordinator	Federal Office for Consumer Protection and Food Safety (BVL)
Test material applied in collaborative trial	Rice flour
Materials used for calibration/controls	Rice flours samples from milled non-GM rice grains and Bt63 rice grains
Tested GM events	
Event Name	Bt63
Unique Identifier	Not applicable
Crop Name	*Oryza sativa* L.

Collaborative Trial Description

Each laboratory received a series of encoded test portions including negative control rice flours and rice noodle flours, 0.1% and 0.05% Bt63 rice flours, and positive "Rice Vermicelli" and "Swallow Sailing Rice Vermicelli" rice noodle flours. Additionally, each participant received three encoded DNA solutions containing four copies, one copy or no copy per µl of the "Bt63" plasmid, respectively. These plasmid DNA samples were analyzed in duplicate. DNA extracted from GM rice sample FR0502519 was provided as positive control DNA. All DNA extracts had to be analyzed in single PCR experiments targeting the cry1Ac/spacer T-nos transgenic construct and the reference gene, respectively.

Method Performance

LOD Relative	≤0.05%	**LOD Absolute**	5 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	1.6%
False negative (%)	0%

Test Level (%)	**0%**	**0.05%**	**0.1%**
Specificity %	Not reported	-	-
Sensitivity %	-	100%	100%

Comment
All rice flours samples were prepared by the ring-trial coordinator. The GM material was obtained from the Chinese Authorities and provided by the EC Joint Research Centre, Ispra.

3. REFERENCES

L. Grohmann, and D. Mäde (2009) "Detection of genetically modified rice: collaborative validation study of a construct-specific real-time PCR method for detection of transgenic Bt rice" Eur Food Res Technol : Vol 228, p. 497–500 (DOI 10.1007/s00217-008-0964-1)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GACTGCTGGAGTGATTATCGACAGA-3'
Target element	*cry1Ac*
Primer Reverse	5'-AGCTCGGTACCTCGACTTATTCAG-3'
Target element	DNA spacer sequences
Amplicon length	83 bp
Probe	5'-FAM-TCGAGTTCATTCCAGTTACTGCAACACTCGAG-TAMRA-3'
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-TTAGCCTCCCGCTGCAGA-3'
Target element	*gos9*
Primer Reverse	5'-AGAGTCCACAAGTGCTCCCG-3'
Target element	*gos9*
Amplicon length	68 bp
Probe	5'-FAM-CGGCAGTGTGGTTGGTTTCTTCGG-Dabcyl-3'
Target element	*gos9* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,30 µmol/L
Primer Rev	0,30 µmol/L
Probe	0,10 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	20”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

Qualitative PCR method for detection of event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	Junction region between the Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and chloroplast-transit-signal peptide (CTP) sequence from Petunia hybrida *epsps* gene
PCR Assay	Single
Detection Chemistry	Agarose gelelectrophoresis
Compendium Reference	SCE/CON/001

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	soybeans
Materials used for calibration/controls	CRM IRMM-410 (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received 5 encoded kernel samples of genetically modified soybean event GTS 40-3-2 and non-transgenic soybean. The quality of the extracted DNA was checked by PCR analysis with primer pair specific for the lectin gene. The detection of the genetic modification was performed with primer pair specific for the 35S promoter and the chloroplast-transit-signal peptide (CTP) from Petunia hybrida *epsps* gene. The identity of the amplicon could be confirmed by Southern blotting with a specific labelled DNA probe or by sequencing.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0 %
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The LOD value has not been determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (1998). Food Analysis, L 23.01.22-1. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGATGTGATATCTCCACTGACG-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-TGTATCCCTTGAGCCATGTTGT-3'
Target element	*CTP4*
Amplicon length	172 bp
GMO Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCATCC-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTTGTG-3'
Target element	*Le1*
Amplicon length	118 bp
Taxon Target element	lectin (*Le1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
AmpliTaq Gold® DNA polymerase	0,5 IU	AmpliTaq Gold® DNA polymerase	0,5 IU
Sterile water	#	Sterile water	#
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	30”		95°C	30”	
Annealing	60°C	30”		60°C	30”	
Extension	72°C	25”		72°C	60”	
Denaturing, Annealing & Extension			35-40			35
Final Extension	72°C	180”	1	72°C	180”	1

Qualitative PCR method for detection of soybean event GTS 40-3-2

1. GENERAL INFORMATION

Target genetic element	Junction region between the Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and the chloroplast-transit peptide (CTP) sequence from Petunia hybrida *epsps* gene
PCR Assay	Single
Detection Chemistry	Agarose gel electrophoresis
Compendium Reference	SC/CON/006

2. VALIDATION DATA

Collaborative trial coordinator	Gene-Scan GmbH
Test material applied in collaborative trial	Soybean flour
Materials used for calibration/controls	CRM (Fluka Chemie AG)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

2.1 Collaborative Trial Description

In the collaborative trial, all laboratories received 0% and 2% GMO samples as control samples and 8 blind samples of soybean flour containing 0%, 0.1%, 0.5%, and 2% of soybean event GTS 40-3-2. The participants used the primer pair GM03/GM04 (targeting the soybean lectin gene (*Le1*)) to control the DNA quality and primer pair p35S-af2/petu-ar1 to identify GMO positive samples. The PCR products were then characterized by restriction enzyme analysis.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	**0%**
False negative (%)	0%

Test Level (%)	0%	0.1%	0.5%	2%
Specificity %	100%	-	-	-
Sensitivity %	-	100%	100%	100%

Comment
No specification was provided in the final report on the effective number of samples analysed for each level. The reported LOD value was not been determined in this collaborative trial.

3. REFERENCES

EU-Project SMT4-CT96-2072. Developments of Methods to Identify Foods Produced by Meansof Genetic Engineering Techniques (DMIF-GEN). Final Report

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TGATGTGATATCTCCACTGACG-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-TGTATCCCTTGAGCCATGTTGT-3'
Target element	*CTP4*
Amplicon length	172 bp
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCATCC-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTTGTG-3'
Target element	*Le1*
Amplicon length	118 bp
Target element	lectin *(Le1)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
Double-distilled sterile water	#	Double-distilled sterile water	#
AmpliTaq Gold® DNA Polymerase	1,0 U	AmpliTaq Gold® DNA Polymerase	1,0 U
PCR Buffer 10x (with 15 mmol/L $MgCl_2$)	1x	PCR Buffer 10x (with 15 mmol/L $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	160 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	160 µmol/L each
Primer Fw	0,60 µmol/L	Primer Fw	0,60 µmol/L
Primer Rev	0,60 µmol/L	Primer Rev	0,60 µmol/L
Template DNA	10 µL	Template DNA	10 µL
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	Temperature	Time	No Cycles	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1	95°C	600”	1
Denaturation	95°C	25”		95°C	30”	
Annealing	62°C	30”		60°C	30”	
Extension	72°C	45”		72°C	60”	
Denaturing, Annealing & Extension			50			35
Final Extension	72°C	420”	1	72°C	180”	1

Qualitative PCR method for detection of tomato event Nema 282F

1. GENERAL INFORMATION

Target genetic element	Junction region between the polygalacturonase (*PG*) gene copy from *Solanum lycopersicum L.* and the nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Single
Detection Chemistry	Agarose gel electrophoresis
Compendium Reference	SC/CON/002

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Raw tomato
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.
Tested GM events	
Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum L.*

2.1 Collaborative Trial Description

In this trial, the genetic alteration present in the GM tomato event Nema 282F was detected using a method that amplifies a region overlapping the inserted polygalacturonase (*PG*) gene copy and the adjacent T-nos terminator. The amplicon identity is confirmed by Southern blotting with a labelled specific DNA probe or by restriction digestion. Participants investigated five coded samples of tomato pulp derived from the unmodified control organism or the genetically modified Zeneca tomato. Additionally one positive and one negative control were provided.

2.2 Method Performance

LOD Relative	not reported	**LOD Absolute**	10 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The reported LOD value was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foods Act (1999). Food Analysis, L 25.03.01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CATCGCAAGACCGGCAACAG-3'
Target element	*T-nos*
Amplicon length	350 bp
Target element	DNA sequence within the junction region

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp (endo) & 180 bp (insert)
Target element	polygalacturonase *(PG)* gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
AmpliTaq Gold® DNA Polymerase	1,0 IU	AmpliTaq Gold® DNA Polymerase	1,0 IU
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Fw	0,40 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,40 µmol/L
Water	#	Water	#
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	94°C	30”	
Annealing	60°C	60”	
Extension	72°C	60”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	360”	1

Qualitative PCR method for detection of maize event MON 810

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MON 810 and the maize host genome
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/EV/001

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	CRM IRMM-413 (JRC-IRMM)
Tested GM events	
Event Name	MON 810
Unique Identifier	MON-00810-6
Crop Name	*Zea mays* L.

Collaborative Trial Description

All laboratories received 5 encoded samples from flour (ground kernels) of maize event MON 810 (0.1%, 1%) and non-GMO maize. Of each sample, two independent DNA extractions were analyzed in duplicate. Quality of the isolated DNA was tested using the maize invertase (Ivr1) gene as a positive control. Confirmation of the PCR products was carried out by restriction analysis.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	0.1%	1%
Specificity %	100%	-	-
Sensitivity %	-	100%	100%

Comment
The reported LOD value was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (2001). Food Analysis, L15.05-01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-TCGAAGGACGAAGGACTCTAACG-3'
Target element	maize host genome
Primer Reverse	5'-TCCATCTTTGGGACCACTGTCG-3'
Target element	*CaMV P-35S*
Amplicon length	170 bp
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CCGCTGTATCACAAGGGCTGGTACC-3'
Target element	*ivr1*
Primer Reverse	5'-GGAGCCCGTGTAGAGCATGACGATC-3'
Target element	*ivr1*
Amplicon length	225 bp
Target element	invertase *(ivr1)* gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR Buffer 10x (without $MgCl_2$)	1x	PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	2 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L	dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Fw	0,50 µmol/L	Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L	Primer Rev	0,50 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 IU	AmpliTaq Gold® DNA Polymerase	1,0 IU
Water	#	Water	#
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

	GM-target(s)			Taxon-target(s)		
Stage	**Temperature**	**Time**	**No Cycles**	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	720”	1	95°C	720”	1
Denaturation	95°C	30”		95°C	30”	
Annealing	64°C	30”		64°C	30”	
Extension	72°C	30”		72°C	60”	
Denaturing, Annealing & Extension			40			35
Final Extension	72°C	600”		72°C	600”	1

Qualitative PCR method for detection of oilseed rape event GT73

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the E9 gene terminator (T-E9) of oilseed rape event GT73 and the oilseed rape host genome
PCR Assay	Single
Detection chemistry	Agarose gelectrophoresis
Compendium Reference	SC/EV/002

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Entry-Exit Inspection & Quarantine Bureau
Test material applied in collaborative trial	oilseed rape meal
Materials used for calibration/controls	Meal derived from dried grain samples of GT73 oilseed rape (Monsanto)
Tested GM events	
Event Name	GT73 (RT73)
Unique Identifier	MON-00073-7
Crop Name	*Brassica napus* L.

2.1 Collaborative Trial Description

Each laboratory received 20 encoded dried meal samples, including 10 samples for the detection of the oilseed rape endogenous high-mobility-group A (*hmgA*) gene and 10 samples for the detection of the exogenous GT73 event-specific fragment. The first series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) non-GM oilseed rape in a rice background. The second series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) GT73 oilseed rape in non-GM oilseed rape samples. The participants were asked to extract DNA from the samples according to the protocol provided. The amplified PCR products were analyzed by electrophoresis. The detection of a DNA fragment with the same size as the positive control indicated that the sample was positive; otherwise the result was considered negative.

2.2 Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	75 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	not reported
False negative (%)	20%

Test Level (%)	**0.01%**	**0.05%**	**0.1%**	**1%**	**5%**
Sensitivity (%)	38%	71%	100%	100%	100%

Comment
The LOD was not determined in this collaborative trial.

3. REFERENCES

L. Pan, S. Zhang, L. Yang, H. Broll, F. Tian, and D. Zhang (2007) "Interlaboratory Trial Validation of an Event-Specific Qualitative Polymerase Chain Reaction-Based Detection Method for Genetically Modified RT73 Rapeseed" Journal of AOAC International, Vol. 90, No. 6, p. 1639-1646

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AATAACGCTGCGGACATCTA-3'
Target element	insert
Primer Reverse	5'-CAGCAAGATTCTCTGTCAACAA-3'
Target element	3'-host genome
Amplicon length	204 bp
GMO Target element	DNA sequence in the IBR

Taxon-target(s)

Primer Forward	5'-GGTCGTCCTCCTAAGGCGAAAG-3'
Target element	*hmgA*
Primer Reverse	5'-GCAACCAACAGGCACCATC-3'
Target element	*hmgA*
Amplicon length	219 bp
Taxon Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

GM-target(s) and Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
Taq DNA Polymerase buffer 10x	1x	Taq DNA Polymerase buffer 10x	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
Taq DNA Polymerase	1,0 U	Taq DNA Polymerase	1,0 U
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	94°C	180”	1
Denaturation	94°C	30”	
Annealing	59°C	30”	
Extension	72°C	40”	
Denaturing, Annealing & Extension			40
Final Extension	72°C	180”	1

Qualitative PCR method for detection of maize invertase gene

1. GENERAL INFORMATION

Target genetic element	Invertase gene (*ivr1*)
PCR Assay	single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/003

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	maize kernels
Materials used for calibration/controls	CRM IRMM-411, CRM IRMM-412 (JRC-IRMM)
Tested GM events	
Event Name	Bt 176 and Bt11
Unique Identifier	SYN-EV176-9 and SYN-BT011-1
Crop Name	*Zea mays* L.

Collaborative Trial Description

The participants received 6 encoded samples consisting of maize Bt-176, Bt11and non-transgenic control maize. Of each sample, two independent DNA extractions had to be analyzed in duplicate. Quality of the isolated DNA was tested with a control primer pair that amplifies a single copy species specific invertase (*ivr1*) gene sequence in maize. Confirmation of the PCR products was carried out by restriction analysis or by Southern blot hybridization.

Method Performance

LOD Relative	not reported	**LOD Absolute**	≤0.1 ng of DNA
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	
Sensitivity %		100%

Comment
The absolute LOD was not determined in the collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modifiedorganisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (2001). Food Analysis, L15.05-01. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-CCGCTGTATCACAAGGGCTGGTACC-3'
Target element	*ivr1*
Primer Reverse	5'-GGAGCCCGTGTAGAGCATGACGATC-3'
Target element	*ivr1*
Amplicon length	225 bp
Taxon Target element	invertase (*ivr1*) gene

5. PCR REACTIONS SETUP

Taxon-target(s)	
Reagent	**Final Concentration**
AmpliTaq Gold® DNA polymerase	1,0 IU
PCR Buffer 10x (without MgCl2)	1x
MgCl2	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Water	#
Primer Fw	0,50 µmol/L
Primer Rev	0,50 µmol/L
Template DNA	10-50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)			
Stage	**Temperature**	**Time**	**No Cycles**
Activation/Initial Denaturation	95°C	720”	1
Denaturation	95°C	30”	
Annealing	64°C	30”	
Extension	72°C	60”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	600”	1

Qualitative PCR method for detection of oilseed rape high-mobility-group A gene

1. GENERAL INFORMATION

Taxon Target element	High-mobility-group A (*hmgA*) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/004

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Entry-Exit Inspection & Quarantine Bureau
Test material applied in collaborative trial	oilseed rape meal
Materials used for calibration/controls	Meal derived from dried grain samples of oilseed rape event GT73 (Monsanto)
Tested GM events	
Event Name	GT73 (RT73)
Unique Identifier	MON-00073-7
Crop Name	*Brassica napus* L.

Collaborative Trial Description

Each laboratory received 10 blind coded dried meal samples for detection of the oilseed rape high-mobility-group A (*hmgA)* endogene. These samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) non-GM oilseed rape in a rice background. The participants were asked to extract DNA from the samples according to the protocol provided. The amplified PCR products were analyzed by electrophoresis. The detection of a DNA fragment with the same size as the positive control indicated that the sample was positive; otherwise the result was considered negative.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	75 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	not reported
False negative (%)	9.2%

Test Level ng	0.01%	0.05%	0.1%	1%	5%
Sensitivity %	67%	88%	100%	100%	100%

Comment
The LOD was not determined in this collaborative trial

3. REFERENCES

L. Pan, S. Zhang, L. Yang, H. Broll, F. Tian, and D. Zhang (2007) "Interlaboratory Trial Validation of an Event-Specific Qualitative Polymerase Chain Reaction-Based Detection Method for Genetically Modified RT73 Rapeseed" Journal of AOAC International, Vol. 90, No. 6, p. 1639-1646

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-GGTCGTCCTCCTAAGGCGAAAG-3'
Target element	*hmgA*
Primer Reverse	5'-GCAACCAACAGGCACCATC-3'
Target element	*hmgA*
Amplicon length	219 bp
Taxon Target element	high-mobility-group A (*hmgA*) gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
Taq DNA Polymerase buffer 10x	1x
$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each
Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L
Taq DNA Polymerase	1,0 U
Template DNA	10-50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	94°C	180”	1
Denaturation	94°C	30”	
Annealing	59°C	30”	
Extension	72°C	40”	
Denaturing, Annealing & Extension			40
Final Extension	72°C	180”	1

Qualitative PCR method for detection of rice sucrose-phosphate synthase gene

1. GENERAL INFORMATION

Target element	Sucrose-phosphate synthase (SPS) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/006

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Jiao Tong University (GMDL-SJTU)
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	Rice varieties (3M, Balilla, Guangluai4, and Shennong265)
Tested GM events	
Event Name	Not applicable
Unique Identifier	Not applicable
Crop Name	*Oryza sativa* L.

Collaborative Trial Description

All laboratories received 12 genomic DNA samples extracted from different rice cultivars for heterogeneity studies and 10 genomic DNA samples extracted from different plant material to validate the species specificity of the sucrose-phosphate synthase (*sps*) gene gene for rice. In addition, the participants received 10 serially diluted DNA samples extracted from powder containing a mixture of maize and varying amounts of rice seeds at 10%, 1%, 0.1%, 0.05% and 0.01% rice content with two repeated samples for assessing the sensitivity of the qualitative PCR method and determining the relative LOD value.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	230 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	1.7%
False negative (%)	0.69%

Test Level (%)	0%	0.01%	0.05%	0.1%	1%	10%
Specificity %	98%	-	-	-	-	-
Sensitivity %	-	17%	33%	100%	100%	100%

Comment
The absolute LOD and LOQ were not determined in this collaborative trial.

3. REFERENCES

L. Jiang, L. Yang, H. Zhang, J. Guo, M. Mazzara, G. Van den Eede, and D. Zhang (2009) "International Collaborative Study of the Endogenous Reference Gene, Sucrose-Phosphate Synthase (SPS), Used for Qualitative and Quantitative Analysis of Genetically Modified Rice" J. Agric. Food Chem. Vol 57, p. 3525–3532 (DOI:10.1021/jf803166p)

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-TTGCGCCTGAACGGATAT-3'
Target element	*SPS*
Primer Reverse	5'-GGAGAAGCACTGGACGAGG-3'
Target element	*SPS*
Amplicon length	277 bp
Target element	sucrose-phosphate synthase (*SPS*) gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
KCl	10 mmol/L
$MgSO_4$	2 mmol/L
$(NH4)_2SO_4$	8 mmol/L
Tris-HCl (pH 8.3-8.8)	10 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	0,20 µmol/L
$MgCl_2$	2,5 mmol/L
Primer Fw	0,33 µmol/L
Primer Rev	0,33 µmol/L
Template DNA	100 ng
Taq DNA Polymerase	1,0 U
Final Volume	not specified

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	900”	1
Denaturation	95°C	30”	
Annealing	58°C	30”	
Extension	72°C	30”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	420”	1

Qualitative PCR method for detection of soybean lectin gene

1. GENERAL INFORMATION

Target genetic element	Lectin (*Le1*) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/002

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and VeterinaryMedicine (BgVV)
Test material applied in collaborative trial	Soybean grain
Materials used for calibration/controls	CRM IRMM-410 (JRC-IRMM)
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories used a primer pair specific for the lectin gene (*Le1*), which is a taxon-specific marker for detection of soybean-derived components. The laboratories received encoded samples of soybean event GTS 40-3-2 and non-transgenic soybean grains. The identity of the amplicons could be confirmed by Southern blotting with a specific labelled DNA probe or by sequencing.

Method Performance

LOD Relative	not reported	**LOD Absolute**	≤0.1 ng DNA
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The reported absolute LOD was not determined in this collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foodstuffs Act (1998). Food Analysis, L 23.01.22-1. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-GCCCTCTACTCCACCCCCATCC-3'
Target element	*Le1*
Primer Reverse	5'-GCCCATCTGCAAGCCTTTTTGTG-3'
Target element	*Le1*
Amplicon length	118 bp
Target element	lectin *(Le1)* gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
PCR Buffer 10x (without $MgCl_2$)	1x
$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	800 µmol/L
Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L
AmpliTaq DNA Polymerase	0,5 IU
Sterile water	#
Template DNA	10-50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	30”	
Annealing	60°C	30”	
Extension	72°C	60”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	180”	1

Qualitative PCR method for detection of tomato LAT52 gene

1. GENERAL INFORMATION

Target genetic element	LAT52 gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/005

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Jiao Tong University (GMDL-SJTU)
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	Genomic DNA samples extracted from four tomato varieties, namely R144, Zhongsu5, Zaofeng and Lichum
Tested GM events	
Event Names	Not applicable
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

Collaborative Trial Description

Each participant received 12 tomato genomic encoded DNA samples extracted from tomato cultivars having different geographic and phylogenic origins, 10 other plant genomic encoded DNAs derived from either evolutionary related species or frequently used plant materials, 10 encoded DNA samples representing double-blind replicates of five concentration levels (2%, 0.5%, 0.1%, 0.05%, and 0.01% (w/w) tomato powder mixed in maize). In addition participants received one positive DNA target control and one negative DNA control consisting of a salmon sperm DNA solution.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	3.1%
False negative (%)	0.6%

Test Level (%)	0	0.01	0.05	0.1	0.5	2
Specificity %	97%	-	-	-	-	-
Sensitivity %	-	15%	0%	100%	96%	96%

Comment
All reference materials were produced by the trail responsible. The specificity of the method was demonstrated on different tomato varieties.

3. REFERENCES

L. Yang, H Zhang,J. Guo, L. Pan, and D. Zhang (2008) "International Collaborative Study of the Endogenous Reference Gene LAT52 Used for Qualitative and Quantitative Analyses of Genetically Modified Tomato' J. Agric. Food Chem., Vol 56, p. 3438–3443

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-AGACCACGAGAACGATATTTGC-3'
Target element	*LAT52*
Primer Reverse	5'-TTCTTGCCTTTTCATATCCAGACA-3'
Target element	*LAT52*
Amplicon length	92 bp
Taxon Target element	*LAT52* gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
PCR Buffer	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	0,20 µmol/L each
Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L
HotStarTaq® DNA Polymerase	1,0 U
Template DNA	5 µL
Final Volume	30 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	94°C	900”	1
Denaturation	94°C	30”	
Annealing	56°C	30”	
Extension	72°C	30”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	420”	1

Qualitative PCR method for detection of tomato polygalacturonase gene

1. GENERAL INFORMATION

Target genetic element	Polygalacturonase (*PG*) gene
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	SC/TAX/001

2. VALIDATION DATA

Collaborative trial coordinator	German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV)
Test material applied in collaborative trial	Tomato pulp
Materials used for calibration/controls	Transgenic and control lines provided by Zeneca Ltd.
Tested GM events	
Event Name	Tomato Nema 282F
Unique Identifier	Not applicable
Crop Name	*Solanum lycopersicum* L.

2.1 Collaborative Trial Description

In this trial, participants received 10 samples of tomato pulp derived from the non-transgenic or the GM Tomato Nema 282F. Additionally one positive and one negative control were provided. For detection of the tomato species, a single copy species-specific DNA sequence from the polygalacturonase (*PG*) gene was used. As the genetically modified tomato in this study also contains a fragment of the *PG* gene in sense direction, an additional 180 bp fragment is expected when applying the PG primer pair. All PCR products were subsequently identified based on size of the amplification product.

2.2 Method Performance

LOD Relative	not reported	**LOD Absolute**	≤0.1 ng DNA
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

Test Level (%)	0%	100%
Specificity %	100%	-
Sensitivity %	-	100%

Comment
The absolute LOD reported in this study has not been determined in the collaborative trial.

3. REFERENCES

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Food Analysis, L 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

Taxon-target(s)

Primer Forward	5'-GGATCCTTAGAAGCATCTAGT-3'
Target element	*PG*
Primer Reverse	5'-CGTTGGTGCATCCCTGCATGG-3'
Target element	*PG*
Amplicon length	384 bp (endo) &180 bp (insert)
Target element	polygalacturonase *(PG)* gene

5. PCR REACTIONS SETUP

Taxon-target(s)

Reagent	Final Concentration
Water	#
$MgCl_2$	1,5 mmol/L
PCR Buffer 10x (without $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	400 µmol/L
Primer Fw	0,40 µmol/L
Primer Rev	0,40 µmol/L
AmpliTaq Gold® DNA Polymerase	1,0 IU
Template DNA	10-50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

Taxon-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	94°C	30”	
Annealing	60°C	60”	
Extension	72°C	60”	
Denaturing, Annealing & Extension			35
Final Extension	72°C	360”	1

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Abstract
In accordance with article 32(1) of Regulation (EC) No 882/2004, the European Union Reference Laboratories for feed and food are responsible, among others, for "providing national reference laboratories with details of analytical methods, including reference methods". In this frame, a "Compendium of Reference Methods for GMO Analysis" was produced jointly by the EURL-GMFF and the ENGL in 2010, aiming at providing a technical state of the art of the detection methods applied in GMO analysis that have been validated according to international standards. The methods collected have been selected based on their reported compliance with ISO 5725 international standard and/or the IUPAC protocol.

Each method has then been described in a comprehensive summary which provides the essential information related to the validated method. Not all details are given for each method but all necessary references are provided for further information about each method. This Compendium contains 79 DNA-based reference PCR detection methods; in follow-up editions, it is foreseen to extend the scope of this "Compendium of Reference Methods for GMO Analysis" to include also protein-based detection methods and extraction methods.

The present document is a revised edition aligning the "Compendium of Reference Methods for GMO Analysis" with the online database released at *http://gmo-crl.jrc.ec.europa.eu/gmomethods/*

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