Regulation (EC) No 641/2004: explanatory notes to applicants

13 October 2008
Date of application: 13 April 2009

This document aims at providing the applicants with practical instructions concerning the method validation task of the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF) as described in Regulation (EC) No 1829/2003 and in the Regulation (EC) No 641/2004 (so called implementing guidelines). The document should be understood as an explanatory note, in which scientific and technical aspects are described by the CRL-GMFF in collaboration with the European Network of GMO Laboratories (ENGL) according to the scientific expertise prevalent in these bodies. The explanatory notes should not be understood as a legally binding text.

1. General explanatory notes concerning the submission

The method documentation requested by the Regulations is part of the official application to be submitted by the applicant to the competent authorities of the relevant Member State. The CRL-GMFF will receive this through an official pathway of the dossier. If the applicant wishes, he may send an advance note of the up-coming validation process to the CRL-GMFF. This is a voluntary arrangement, but welcomed by the CRL-GMFF in order to manage the work flow.

The CRL-GMFF wishes to receive the relevant samples (referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003) directly from the applicant in order to guarantee the quality and integrity of the samples. The samples should be sent to:

European Commission - Joint Research Centre
Institute for Health and Consumer Protection
Unit "Biotechnology and GMOs"
Unit Head: Mr Guy Van den Eede
TP 331 Via Fermi 2749
I-21027
Ispra (VA), ITALY

The parcel should be specified to contain “Free samples”, and it should include the list of all items and their storage instructions clearly indicated. If the samples contain genomic DNA these should be shipped in dry ice. In addition, it is recommended to send an advance notice of the arriving delivery (e.g. at the time of shipment) to: marco.mazzara@jrc.it

2. Notes to the Article 32 of the Regulation (EC) No 1829/2003

The Article 32 states that “Applicants for authorization of genetically modified food and feed shall contribute to supporting the costs of the tasks of the Community reference laboratory and the European Network of GMO laboratories mentioned in the Annex. The contributions from applicants shall not exceed the costs incurred in carrying out the validation of detection methods”
This article is implemented by Commission Regulation (EC) No 1981/2006.

The JRC has already invested and will continue to invest in the establishment and maintenance of the CRL-GMFF. This involvement includes the laboratory space, necessary equipment, appropriate levels of staffing and total quality assurance management according to ISO 17025:2005 and ISO 9001:2000. In addition, the CRL-GMFF will carefully record the costs, which occur from the direct handling of a particular dossier (e. g. personnel, working time, consumables and subcontracting the experimental work to the participating laboratories). These records allow the allocation of the direct costs and the verification of the applicant contribution related to a certain dossier.
The three main causes of the direct costs of a validation process are the specific reagents, experimental work in the participating laboratories and the working time dedicated to the scientific assessment and laboratory activities in the CRL-GMFF. Commission Regulation (EC) No 1981/2006 details the amount of the costs generated according to the type of method submitted by the applicant.

3. Notes to Article 30 of the Regulation (EC) No 1829/2003

The article 30(3)f states that the information related to the "methods 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 referred to in Articles 3(1) and 15(1)" shall not be considered confidential. In addition, Article 30(5) states "The use of detection methods and the reproduction of the reference materials, provided under articles 5(3) and 17(3) for the purpose of applying this Regulation to the GMOs, food or feed to which an application refers, shall not be restricted by the exercise of property rights."

The CRL-GMFF aims at making the method available to the official ENGL members after the validation process has been concluded successfully. At this stage the CRL-GMFF will make the method (i.e. validation reports and validated method) publicly available at http://gmo-crl.jrc.ec.europa.eu

Should the applicant wish to make the method publicly available during the validation process he should notify the CRL-GMFF about this in writing. Accordingly, the method will be made available, either directly or via a link to a relevant site provided by the applicant at http://gmo-crl.jrc.ec.europa.eu

4. Explanatory notes to the Annex I of the Regulation (EC) 641/2004

Annex I	Explanatory note
A. For the purpose of implementing Articles 5 (3) (i) and 17 (3) (i) of Regulation (EC) No 1829/2003, this annex provides guidelines 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. All guidance documents referred to in this annex or produced by the Community Reference Laboratory (CRL) shall be made available by the CRL.	The CRL-GMFF makes the guidance documents available and updated at http://gmo-crl.jrc.ec.europa.eu/
B. The method acceptance criteria and method performance requirements have been compiled by the European Network of GMO Laboratories (ENGL) in a document entitled "Definition of minimum performance requirements for analytical methods of GMO testing", which shall be made available by the CRL. "Method acceptance criteria" are criteria, which should be fulfilled prior to the initiation of any method validation by the CRL. The "method performance requirements" define the minimum performance criteria that the method should demonstrate upon completion of a validation study carried out by the CRL according to internationally accepted guidelines and this in order to certify that the method validated is fit for the purpose of enforcement of Regulation (EC) No 1829/2003.	The method acceptance criteria and method performance requirements are available at: http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm It should be noted that these criteria and requirements represent the current scientific understanding of the methods and are aimed to be advisory for the current methodology, legal thresholds and standard applications. In a specific case it may be justified to deviate from the said limits.
C. The CRL, established under Regulation (EC) No 1829/2003 and assisted by ENGL, will evaluate the provided information for its completeness and fitness for the purpose. Here, the method acceptance criteria recommended by ENGL, which are described under 1(B), will be taken into account.	This is done during the scientific assessment of the validation process (step 2).
D. If the information provided about the method is considered adequate and fulfils the method acceptance criteria, the CRL will initiate the validation process for the method.	This means that the step 2 (i.e. scientific assessment) has been completed successfully and that the validation process can continue with the CRL-GMFF experimental testing (step 3).

Annex I	Explanatory note
E. The validation process will be carried out by the CRL according to internationally accepted guidelines.	The validation process is carried out under ISO 17025 requirements ("General requirements for the competence of testing and calibration laboratories"). Moreover the CRL-GMFF takes the following documents in consideration as appropriate: – Horwitz, W. (1995) Protocol for the design, conduct and interpretation of method performance studies, Pure and Appl. Chem, 67, 331-343; – ISO 5725: Accuracy (trueness and precision) of measurement methods and results. Foodstuffs – Methods of analysis for the detection of GMOs and derived products: – General requirements and definitions: ISO 24276; – Nucleic acid extraction: ISO 21571; – Quantitative nucleic acid based methods: ISO 21570; – Protein based methods: ISO 21572; – Qualitative nucleic acid based methods: ISO 21569.
F. The CRL, together with ENGL, shall provide further information about the operational procedures of the validation process and shall make the documents available.	This information is provided at http://gmo-crl.jrc.ec.europa.eu, and the CRL-GMFF can be contacted for further clarifications.
G. The CRL, assisted by ENGL, shall evaluate the results obtained in the validation study for the fitness for the purpose. Here, the method performance requirements as described under 1(B) shall be taken into account.	The CRL-GMFF will evaluate the results. If the results are within the requirements, the reporting will commence. If specific scientific interpretation of the results is required, the CRL-GMFF main consult the ENGL and/or the applicant.

Annex I

2. INFORMATION ABOUT THE METHOD

A. The method shall refer to all the methodological steps needed to analyse the relevant material in accordance with Articles 5 (3) (i) and 17 (3) (i) of Regulation (EC) No 1829/2003. For a particular material this must include the methods for DNA extraction and the subsequent quantification in a Polymerase Chain Reaction (PCR) system. In such a case, the whole process from extraction up to the PCR-technique (or equivalent) constitutes a method. The applicant shall provide information about the whole method.

Explanatory note

PCR method

The proposed method should not be a commercial kit or alike. It should be possible to purchase the individual method components separately (e.g. master-mix and different primers and probes) and then carry out the testing. The necessary chemicals and laboratory equipment to be used should be easily accessible and publicly available. Commonly, official or reference methods may be lengthier or more complicated to execute than commercial kits, but are set to provide reliable results and to remain executable for a long time (a kit may be withdrawn from the market any time). The results from different commercial kits can be calibrated against the officially validated methods.

The technology selected should allow the specific and reproducible detection of the genetically modified (GM) analyte and the related parental non GM analyte and to quantify their ratio.

Analyte extraction/purification method

The proposed method should not entirely rely on the use of a commercial kit or alike. If the use of commercial kits is proposed, the applicant should provide justification and clear experimental evidence supporting the choice (e.g. significant improvement of the analyte quality, significant gain of time or cost reduction). Should a method refer to a commercial kit that is at a later stage withdrawn from the market, the applicant shall provide an alternative analyte extraction/purification method. The method should be proposed together with the list of matrices to which the method is applicable.

Sample preparation

The detailed protocol and materials needed for sample preparation (e.g. grinding, homogenization) shall be provided as part of the whole method.

B. As described in the document referred to under 1(B), ENGL recognises the modularity of a method. According to this principle, the applicant is allowed to refer to existing methods for a certain module(s), if available and appropriate. This could be, for instance, a DNA extraction method from a certain matrix. In such a case, the applicant shall provide experimental data from an in-house validation in which the method module has been successfully applied in the context of the application for authorisation.

A method or methods allowing isolating and/or concentrating and purifying the analytes shall proposed together with the list of matrices, which illustrate the applicability of the method. If the applicant wishes to refer to an existing and documented method (e.g. PCR or DNA extraction methods) he may do so and demonstrates, as described below, the performance of the method in the context of the current application (in-house testing, acceptance criteria of ENGL documents described under 1.B).

There are several DNA extraction and GM detection methods available and in wide use, which have not undergone a proper validation study. Should these be proposed by the applicant, it is up to the CRL-GMFF, assisted by the ENGL, to decide on a case-by-case basis whether a full collaborative study is needed.

Annex I	Explanatory note
C. The applicant shall demonstrate that the method fulfils the following requirements:	
(1) The method shall be event-specific and thus must only be functional with the GMO or GM based product considered and shall not be functional if applied to other events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised events and conventional counterparts, in the case of GM plants. This testing shall include closely related events, where relevant, and cases where the limits of the detection are truly tested. The same specificity principle must be applied for products that consist of or contain GMOs other than plants.	The applicant shall demonstrate testing results on specificity of the event-specific assay with at least: - All the GM events, which are authorised for the applicant in different parts of the world and in the applicant pipeline (i.e. in development); - All the GM events for which there are official reference materials available at the date of deposit of the application for authorisation. It is recommended to test the specificity also with such events which are in the production pipe-ine of the applicant and with the events from other GM products, where available - The event itself and its isogenic line (negative control) with reference to the breeding tree.
(2) The method shall be applicable to samples of the food or feed, to the control samples and to the reference material, which is referred to in Articles 5 (3) (j) and 17 (3) (j) of Regulation (EC) No 1829/2003.	The applicant shall provide testing results of the whole method submitted (i.e. from sample preparation to GMO quantification) on samples submitted in the context of the application (including, where available, reference materials). Testing results shall comply with the ENGL document described under 1(B). The CRL-GMFF will test the method with the control samples and samples of food and feed and with reference material should this be available during the validation process.
(3) The method shall be developed taking the following documents in consideration as appropriate: – General requirements and definitions: standard ISO 24276; – Nucleic Acid extraction ISO 21571; – Quantitative nucleic acid based methods: ISO 21570; – Protein based methods: Adopted European standard ISO 21572;	The described standards have been published as international standards (references below) and are aimed to serve as instructions to the applicants during method development, in-house testing and for documentation and data submitted in the context of the application. Foodstuffs – Methods of analysis for the detection of GMOs and derived products: – General requirements and definitions: ISO 24276;

– Qualitative nucleic acid based methods: standard ISO 21569.

– Nucleic acid extraction: ISO 21571;
– Quantitative nucleic acid based methods: ISO 21570;
– Protein based methods: ISO 21572;
– Qualitative nucleic acid based methods: ISO 21569.

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D. For the purpose of implementing Articles 5 (3) (i) and 17 (3) (i) of Regulation (EC) No 1829/2003, the applicant shall provide:

(a) in the case of an application for authorisation covering a GMO, products consisting of or containing a GMO or products produced from a GMO, the event-specific quantitative detection method of the GM material;

(b) in addition, in the case of an application for authorisation covering products produced from a GMO where the genetically modified material is detectable, the event-specific quantitative detection method in the foods or feeds produced from the GMO.

Explanatory note

The method is foreseen to consist, considering the current practice, of a module for DNA extraction applicable to the relevant matrices and a module for the subsequent quantification in a real-time Polymerase Chain Reaction (PCR) system

The Annex text is understood to refer to the availability of a method to analyse the matrix relevant for a particular notification.

Example: If, for instance, papaya seeds are notified (case (a) of the annex text), the applicant should provide a method, which is applicable to seeds. If, in contrast, the applicant has notified a papaya marmalade and the GM elements are detectable in the marmalade (case (b) of the annex text), then the applicant should provide a method, which is applicable to papaya marmalade. In practise, the GM detection module is likely to be similar in both cases but the method to extract the analyte may be different.

The scope of the application shall be clearly indicated to the CRL-GMFF

Annex I

E. The applicant shall provide a complete and detailed description of the method. The following points shall be clearly addressed:

(1) Scientific basis: An overview of the principles of how the method works, such as DNA molecular biology based (e.g. for real-time PCR) information must be provided. It is recommended to provide references to relevant scientific publications.

(2) Scope of the method: Indication of the matrix (e.g., processed food, raw materials), the type of samples and the percentage range to which the method can be applied.

(3) Operational characteristics of the method: The required equipment for the application of the method shall be clearly mentioned, with regard to the analysis per se and the sample preparation. Further information of any specific aspects crucial for the application of the method shall also be mentioned here.

(4) Protocol: The applicant shall provide a complete optimised protocol of the method. The protocol shall present all the details as required to transfer and apply the method independently in other laboratories. It is recommended to use a protocol template, which can be obtained from the CRL-GMFF. The protocol shall include details of:

– analyte to be tested;

– working conditions, instructions and rules;

– all the materials needed, including an estimation of their amounts and storage and handling instructions;

– all the equipment needed, including not only the main equipment such as a PCR system or centrifuge but also small items such as micropipettes and reaction tubes with an indication of their appropriate sizes, etc.;

Explanatory note

The text of the Annex is understood to be self-explanatory.

– all the steps of the operative protocol, clearly described;
– instructions for the data recording (e.g. the programme settings or parameters to be included).

(5) The prediction model (or alike) needed to interpret results and to make inferences must be described in full details. Instructions for the correct application of the model should be provided.

Annex I	Explanatory note
3. INFORMATION ABOUT THE METHOD TESTING CARRIED OUT BY THE APPLICANT	
A. The applicant shall provide all the available and relevant data of the method optimisation and testing carried out. These data and results shall be presented, where possible and appropriate, by using the performance parameters recommended by the ENGL as referred to under 1(B). A summary of the testing carried out and the main results as well as all the data including the outliers shall be provided. The CRL, together with ENGL, shall continue to provide further guidelines about the appropriate formats for these data.	Format for the submission of the data are provided at: http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm The applicant should provide details on the preparation and measurement of the unknown samples (including number of replicates per test result). The applicant may be asked to provide raw data of experiments. Data shall be generated using DNA extracted with the DNA extraction protocol submitted in the context of the dossier Data should be provided for control samples, food and feed samples and where applicable for reference materials.
B. The information provided shall demonstrate the robustness of the method for inter-laboratory transferability. This means that the method should have been tested by at least one laboratory that is independent from the laboratory which has developed the method. This is an important pre-condition for the success of the validation of the method.	It is recommended that the additional testing laboratory is not a laboratory within the same organisation which has developed the method. The sole aim of this recommendation is to make sure, as far as possible, that the method protocol is fully optimised and properly documented to allow inter-laboratory transfer and to increase the chance of success of a collaborative study. The applicant shall indicate in the testing results the results obtained by different laboratories. The applicant may be requested to provide raw data corresponding to the experiments performed in the alternate laboratories.

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C. Information required about the method development and the method optimisation:

(1) Primer pairs tested (in the case of a PCR-based test): justification shall be given of how and why the proposed primer pair has been selected;

(2) Stability testing: experimental results from testing the method with different varieties shall be provided.

(3) Specificity: the applicant shall submit the full sequence of the insert(s), together with the base pairs of the host flanking sequences needed to establish an event-specific detection method. The CRL shall enter these data in a molecular database. By running homology searches, the CRL will thus be in a position to assess the specificity of the proposed method.

Explanatory note

In general, the method shall express the result as a percentage that is calculated by dividing for a particular species, the amount of the GM species by the amount of the non-GM species in the sample and multiplied by 100. Therefore, the amount of GMO has to be measured by quantifying an unambiguous GM-specific target (event-specific assay) and the amount of a non-GM species-specific target (taxon-specific assay)

The applicant shall provide the base pairs of the host flanking sequences needed to establish an event-specific detection method.

Sequences should be provided according to the explanatory note "guideline for the submission of DNA sequences to the CRL-GMFF" available at http://gmo-crl.jrc.ec.europa.eu/

Species-specific target

In a PCR-based test, the species-specific target should possibly be a single-copy DNA region present in only one allele for the locus within the species, across a globally representative and diverse sample of the species variety. The species-specific assay should be quantitatively stable in the different genetic backgrounds in order to allow stable testing results The applicant shall demonstrate the copy number of the species-specific target per genome and the zygosity of the target sequence.

The choice of the species-specific target shall be documented by the applicant, by providing an EMBL/GenBank accession number as well as a blast search against major databases providing theoretical evidence of the specificity of the species-specific target within the species and justification of the choice of species-specific assay (primers and probe). The specificity of the species-specific assay shall be tested on species related to the one subject of the application. Those species shall be of interest for the food and feed chain. For example, if GM-peas are the scope of the application for authorisation, the species-specific assay should be tested on other species of leguminosae such as lentils, beans,

etc.

The applicant shall provide the sequence of species-specific target and its accession number if available.

Sequences should be provided according to the explanatory note “guideline for the submission of DNA sequences to the CRL-GMFF” available at http://gmo-crl.jrc.ec.europa.eu/

The CRL-GMFF will store the sequence information into a molecular database, which is not accessible through the internet in order to secure the confidentiality.

The event-specific assay shall unambiguously detect the GM-specific target within a mixture of other GM ingredients as well as in non-GM ingredients. For specificity tests of the event-specific assay refer to section 2.C.(1).

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D. Testing report. Besides the values obtained for the performance indices, the following information regarding the testing shall be provided, as appropriate:

– Participating laboratories, time of the analysis and outline of the experimental design, including the details about the number of runs, samples, replicates etc.;

– Description of the laboratory samples (e.g. size, quality, date of sampling), positive and negative controls as well as reference material, plasmids and alike used;

– Description of the approaches that have been used to analyse the test results and outliers;

– Any particular points observed during the testing;

– References to relevant literature or guidelines used in the testing.

Explanatory note

The text is understood to be mainly self-explanatory, and the applicant should seek assistance from what is mentioned under point C.3.

Additional practical examples (refer also to section 3.A):

the standard curve should cover at least the dynamic range (see method acceptance criteria and method performance requirements), but it can also be wider (e.g. 0 – 10% GM); it is recommended to construct the standard curve with at least 4 – 5 different concentrations;

the standard curve should be used to quantify several unknown samples distributed throughout the whole dynamic range. The applicant may, for instance, use unknown samples at five different concentrations (e.g., 0.09%, 0.5%, 0.9%, 2%, and 4.5. Adequate controls should be used throughout.

It is recommended to quantify standard samples as on average of 3 – 4 reaction repetitions.

Annex I	Explanatory note
4. SAMPLES OF THE FOOD AND FEED AND THEIR CONTROL SAMPLES	The applicant shall provide sufficient amount of samples to allow the CRL-GMFF to fulfill its duties and tasks according to the Annex to Regulation (EC) No 1829/2003 and its subsequent amendment in Annex III to Commission Regulation (EC) No 1981/2006, particularly in what refers to the distribution to national reference laboratories of the appropriate positive and negative control samples and to the testing and validation of the detection method. It is the responsibility of the applicant to verify that the material amounts are sufficient. The necessary amounts depend on the proposed method and therefore the CRL-GMFF cannot determine the detailed amounts in a generic manner. Should the applicant wish to receive more detailed information for a specific case, he should approach the CRL-GMFF with a request and provide the full method information to allow the CRL-GMFF to do the necessary calculations. The applicant should appropriately package the materials to prevent any cross-contamination between the positive and negative samples, the degradation caused by temperature and other external shocks, the spoiling of materials caused by microbiological agents, or any other damage during the shipment. The material shall be listed in an accompanying note, including instructions for storage conditions and specific handling of the material, where appropriate.

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In view of implementing Articles 5 (3) (j) and 17 (3) (j) of Regulation (EC) No 1829/2003, the applicant shall, together with the information specified under sections 1, 2 and 3, also provide samples of the food and feed and their control samples of a type and amount to be specified by the CRL for the specific application for authorisation.

Explanatory note

Control samples

Control samples to be provided by the applicant consist of:

- A positive control
- A negative control

The positive and negative control samples will be used for the validation of the detection module of the method (e.g. quantitative real-time PCR). Control samples are defined in the Regulation (EC) 1829/2003 as GMO or its genetic material. The specific control can be used by the CRL-GMFF for additional tests (e.g. specificity). The CRL-GMFF recommends the provision of genetic material, i.e. genomic DNA. The purity and quality of the DNA should be at levels, which allow an accurate amplification of the targets.

Positive control

The positive control should be derived from plant material that contains 100% of the GM event that is the subject of the application (if not 100%, the purity of the positive control shall be demonstrated by the applicant). The positive control sample shall not contain any other GM event or other material belonging to a different species

Negative control

The negative control should contain the same genetic background of the positive control except for the insertion region of the transgene. This means that the genetic difference between the negative and positive controls is restricted as much as possible to the locus of insertion of the transgenic vector.

Examples: If the GM event is the result of a breeding process, the nearest isogenic wild type shall be provided as a negative control sample. In addition the detailed breeding steps should be documented. If the GM event is the primary transformation event, the plant material that was subject of the transformation process should be selected as negative control samples as well as specific control.

The negative control samples shall not contain any GM-event or other material belonging to a different species

It is required that the positive and negative controls have the same ploidy level.

The GM plant material used to prepare DNA should be preferentially homozygous for the transgene insert. If the DNA is derived from seeds, the GM seeds should be homozygous for the transgene insert, in order to avoid problems e.g. due to maternal effects. If it is technically or biologically impossible to obtain homozygous plant material it is requested to submit leaf material or the genetic material isolated thereof.

Samples of Food and Feed

The samples of food and feed are used to validate the method related to the analyte extraction. For the purposes of the CRL-GMFF, one type of matrix which is relevant in the context of the application will suffice as food or feed sample. This sample should be as close as possible to the material for which the authorisation of the event has been submitted. Depending on the application, this can be for instance seeds, grain, flour or papaya marmalade.

The food or feed sample should contain the GM event of the application, and the applicant should report the GM concentration present in the sample. The product should contain the GMO at a minimum content of 0.9% (or other relevant legal labelling threshold).
If the analytes used in the detection method are not significantly present in the material at the 0.9% level, the first material upstream in the processing that allows the extraction of appropriate amount of the analyte should be submitted as food and/or feed sample. The applicant shall justify such a selection made.

For example, if rapeseed oil, which does not contain any GM material at a detectable level, is the object of the application, the CRL-GMFF recommends to submit seeds or grains as food or feed sample, depending on the prevalent processing procedures.

The applicant should provide instructions for the homogenisation of the food and feed sample.

5. Acronyms and definitions

- *Applicability*: the description of analytes, matrices and concentrations to which the method can be applied.

- *Collaborative Trial or Interlaboratory Study*: a study in which several laboratories measure a quantity in one or more identical portions of homogeneous, stable materials under documented conditions, the results of which are compiled into a single document. Guidelines for performing collaborative trials are elaborated by ISO 5725 and ISO/AOAC/IUPAC harmonized protocol.
- *Control sample*: the GMO or its genetic material (positive sample) and the parental organism or its genetic material that has been used for the purpose of the genetic modification (negative sample).
- *CRL-GMFF*: Community reference Laboratory for Genetically Modified Food and Feed
- *DNA and RNA extraction*: separation of DNA and RNA from the other components in a test sample.

- *Dynamic Range*: the range of concentrations over which the method performs in a linear manner with an acceptable level of trueness and precision

- *EFSA:* European Food Safety Authority

- *ENGL*: European Network of GMO Laboratories

- *Genetically modified organism or GMO*: a genetically modified organism as defined in Article 2(2) of Directive 2001/18/EC, excluding organisms obtained through the techniques of genetic modification listed in Annex I B to Directive 001/18/EC.

- *ISO*: International Standards Organisation.

- *JRC*: The European Commission's Joint Research Centre

- *Laboratory* sample: a sample intended for laboratory inspection or testing.

- *Laboratory*: body that calibrates and/or tests.
- *Limit of detection (LOD)*: 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 collaborative trial or single-laboratory validation.

- *Limit of quantification (LOQ)*: the limit of quantification of an analytical procedure is the lowest amount or concentration of analyte in a sample which can be quantified with an acceptable level of precision and accuracy

- *Linearity*: the linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

- *Measurement uncertainty*: a parameter associated with the result of a measurement, which characterises the dispersion of the values that could reasonably be attributed to the analyte.

- *Method acceptance criteria*: criteria which have to be fulfilled by the method in order to be accepted for a full collaborative trial by the CRL-GMFF. The method acceptance criteria have been defined within the ENGL Network.

- *Method performance requirements*: the minimum performance requirements that the method should demonstrate upon completion of a validation study carried out by the CRL-GMFF according to internationally accepted guidelines and this in order to certify that the method validated is fit for the purpose of enforcement by Regulation (EC) No 1829/2003.

- *PCR*: Polymerase chain reaction

- *PCR quality DNA*: a DNA template of sufficient quality to be amplified by PCR.

- *Practicability*: the ease of operations, the feasibility and efficiency of implementation, the associated unitary cost (e.g. cost/sample) of the method.

- *Precision*: the variability among repeated tests under specified conditions.

- *Reference material*: a material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials {ISO Guide 30: 1992 - 2.1, amended}.

- *Relative Repeatability Standard Deviation (RSD_r)*: 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.

- *Replicate tests*: an analysis of a laboratory sample, Certified Reference Material or Reference Material can be performed more than once, the result of each individual analysis is a replicate test result. The replication can occur at any step in the procedure from laboratory sample, to test sample, to test portion, to test solution to aliquot.

- *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.

- *Robustness*: the robustness of a method is a measure of its capacity to remain unaffected by small, but deliberate deviations from the experimental conditions described in the procedure.

- *Sample*: any material brought into the laboratory for analysis.

- Selectivity: the extent to which the analytical method can determine particular analyte(s) in a complex mixture without interference from the other components in the mixture. A method which is perfectly selective for an analyte or a group of analytes is said to be specific.

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

- *SOP:* standard Operating Procedure.

- *Specificity*: property of a method to respond exclusively to the characteristic or analyte of interest.

- *Target taxon*; the taxon to which the genetically modified organism belongs.

- *Trueness*: the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.

- *Validation:* confirmation by examination and provision of objective evidence that the particular requirement for a specified end use are fulfilled.