ANNEX III

VALIDATION OF METHODS FOR THE DETECTION, IDENTIFICATION AND QUANTIFICATION OF THE TRANSFORMATION EVENT, AND REQUIREMENTS FOR CONTROL SAMPLES AND THE CERTIFIED REFERENCE MATERIAL

1. INTRODUCTION

1. For the purposes of implementing Article 5(3)(i) and (j) and Article 17(3)(i) and (j) of Regulation (EC) No 1829/2003, this Annex sets out requirements on:

(a) the performance characteristics of the submitted method(s);

(b) technical requirements regarding the type of information that the applicant must submit so as to verify that those requirements are met;

(c) samples of the food and feed and their control samples;

(d) certified reference material.

2. The applicant must include information on the method as such and on the method testing carried out by the applicant.

3. The applicant shall also consider further guidance and information about the operational procedures of the validation process that is made available by the EU Reference Laboratory (EURL) as referred to in Article 32 of Regulation (EC) No 1829/2003, assisted by the European Network of GMO Laboratories[(1)].

2. DEFINITIONS

For the purpose of this Annex, the following definitions shall apply:

(a) ‘certified reference material’ means reference material as referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003 and corresponds to any material or substance, one or more of whose property values are certified for calibration or quality control of methods. It is accompanied by a certificate that provides value of the specified property, its associated uncertainty and a statement of metrological traceability;

(b) ‘method performance requirements’ means the minimum performance criteria that the method shall demonstrate upon completion of the validation study carried out by the EURL, according to internationally accepted technical provisions.

3. METHOD VALIDATION

3.1. **Information about the method**

A. The method(s) shall refer to all the methodological steps needed to analyse the relevant food and feed material in accordance with Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003.

For a particular food or feed material, the methodological steps shall include the methods for DNA extraction and the subsequent quantification in a real-time Polymerase Chain Reaction (PCR) System. In such a case, the whole process from extraction up to the PCR-technique shall constitute a method. The applicant shall provide information about the whole method.

B. The applicant shall be allowed to refer to validated protocols, if available and appropriate, for method modules used in the analytical procedure such as a DNA extraction protocol from a certain matrix.

In that 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.

C. The applicant shall demonstrate that the method(s) fulfils the following requirements:

1. The method(s) shall be specific to the transformation event (hereafter referred to as 'event-specific') and thus shall only be functional with the genetically modified organism or genetically modified based product considered and shall not be functional if applied to other transformation 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 transformation events and conventional counterparts. This testing shall include closely related transformation events.

2. The method(s) shall be applicable to samples of the food or feed, to the control samples and to the certified reference material.

3. The applicant shall take into consideration the following documents for the development of the detection method:

   (a) Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions: ISO 24276;

   (b) Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction: ISO 21571;

   (c) Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods: ISO 21570;

   (d) Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods: draft European standard ISO 21569.

4. The method shall also take into consideration the more detailed requirements set out in the common criteria set by the EURL, and the ENGL for minimum performance requirements for analytical methods for GMO testing. These criteria are part of the guidance provided by the EURL.

D. For the purpose of implementing Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003, the applicant shall provide the event-specific quantitative detection method(s) of the genetically modified material. The applicant shall discuss the validity and limitations of the detection methods in the various types of foods and feeds (the various matrixes) that are expected to be placed on the market.

E. The applicant shall provide a complete and detailed description of the method.

The following points shall be clearly addressed by the applicant:

1. Scientific basis: the applicant shall provide an overview of the principles of how the method works. This overview shall include references to relevant scientific publications.

2. Scope of the method: the applicant shall indicate the matrix(es) (for example, processed food, raw materials), the type of samples and the percentage range to which the method may be applied.

3. Operational characteristics of the method: the required equipment for the application of the method shall be specified, with regard to the analysis as such and the sample preparation. Further information of any specific aspects crucial for the application of the method shall also be included.

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.

5. A prediction model (or a similar tool) needed to interpret results and to make inferences shall be described in full details. Instructions for the correct application of the model shall be provided by the applicant.

6. Breeding schemes that are to be applied for the production of genetically modified food and feed and their impact on the interpretation of results shall be provided by the applicant.

3.2. **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 as referred to under point 3.1.C.4. The applicant shall also provide a summary of the testing carried out and the main results as well as all the data including the outliers.

B. The applicant shall ensure that the provided information demonstrates the robustness of the method for inter-laboratory transferability. For this purpose, the applicant shall provide the results of the testing of the method by at least one laboratory that is different from the laboratory which has developed the method.

C. The applicant shall provide the following information about the method development and the method optimisation:

1. primer pairs tested and probe, if appropriate, including a justification as to how and why the proposed primer pair has been selected;

2. stability testing, which shall be established through the submission of experimental results from testing the method with different plant varieties;

3. specificity, which shall be established through the submission of the full sequence of the insert(s) in a standardised electronic format, together with the base pairs of the host flanking sequences so as to enable the EURL to assess the specificity of the proposed method by running homology searches in a molecular database;

4. precision, the relative repeatability standard deviation shall be less than or equal to 25 % related to mass fraction over the whole dynamic range of the method.

D. The applicant shall, in addition to the information required under Sections A, B and C provide the following information regarding the testing:

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

2. description of the laboratory samples (such as size, quality, date of sampling), positive and negative controls as well as certified reference material, plasmids and alike used;

3. description of the approaches that have been used to analyse the test results and outliers;

4. any particular points observed during the testing;

5. references to relevant literature or technical provisions used in the testing.

3.3. **Samples of the food and feed and their control samples**

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

The information accompanying the control samples shall include information on the breeding of the plant which has been used for the production of the control samples and on the zygosity of the insert(s).

The applicant may use the same raw material for the production of certified reference material and for the production of control samples.

4. CERTIFIED REFERENCE MATERIAL

The certified reference material shall be produced under ISO Guide 34 (General requirements for the competence of reference material producers) by a producer accredited to ISO Guide 34.

The applicant shall provide information as regards the place where the certified reference material can be accessed. This shall be accompanied by adequate information demonstrating that the availability of the certified reference material will be maintained throughout the period of validity of the authorisation. For verification and value assignment, a method that has been properly validated (see ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories) shall be used.

Uncertainties shall be estimated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM).

The main characteristics of those internationally accepted technical provisions are the following:

1. Genetically modified reference material containers:

   (a) genetically modified reference material container (such as bottles, vials, ampoules) shall be tight and contain not less than the stated amount of material;

   (b) the commutability of the genetically modified reference material must be assured;

   (c) packaging shall be appropriate to the purpose;

   (d) labelling shall be of good aspect and quality.

2. Homogeneity testing:

(a) samples shall have appropriate homogeneity;

(b) between-bottle homogeneity shall be examined;

(c) any possible between-bottle heterogeneity shall be accounted for in the overall estimated reference material uncertainty. This requirement shall apply even when no statistically significant between-bottle variation is present. In this case, the method variation or the actual calculated between-bottle variation, whichever is larger, shall be included in the overall uncertainty.

3. Stability testing:

(a) samples shall have appropriate stability;

(b) stability shall be positively demonstrated by appropriate statistical extrapolation for the genetically modified reference material shelf-life to be within the stated uncertainty; the uncertainty related to this demonstration is part of the estimated reference material uncertainty. Assigned values are valid only for a limited time and shall be subject to a stability monitoring.

4. Batch characterisation:

1. The methods used for verification and for certification shall:

(a) be applied under metrologically valid conditions;

(b) have been properly technically validated before use;

(c) have precision and trueness compatible with the target uncertainty.

2. Each set of measurements shall:

(a) be traceable to the stated references;

(b) be accompanied by an uncertainty statement whenever possible.

3. Participating laboratories shall:

(a) have the required competence for the execution of the task;

(b) be able to achieve traceability to the required stated references;

(c) be able to estimate their measurement uncertainty;

(d) have in place a sufficient and appropriate quality assurance system.

5. Final storage:

1. To avoid degradation after sample production, all samples shall be stored under conditions designated for the final storage of the genetically modified certified reference material before measurements are started.

2. Otherwise, they shall be transported from door to door keeping them at all times under such storage conditions for which it has been demonstrated that there is no influence on the assigned values.

6. Establishment of a certificate for certified reference material:

A certificate complemented by a certification report shall be established, containing all information relevant to and needed by the user.

The certificate and report shall be made available when the genetically modified certified reference material is distributed.

The information accompanying the certified reference material shall include information on the breeding of the plant which has been used for the production of the certified reference material and on the zygosity of the insert(s).

The certified value of the GMO content shall be given in mass fraction and, where available, in copy number per haploid genome equivalent.

Certified values (such as quantity of genetically modified material expressed in mass fraction) shall be traceable to stated references and be accompanied by an expanded uncertainty statement valid for the entire shelf-life of the genetically modified certified reference material.

(**1**) http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

Changes to legislation:
There are outstanding changes not yet made to Commission Implementing Regulation (EU) No 503/2013. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to :

- Annex 3 words substituted by S.I. 2019/705 reg. 56(b)

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by S.I. 2019/705 reg. 53
- Annex 1 Pt. 7 para. 1.1(a) omitted by S.I. 2019/705 reg. 54(e)(iii) (This amendment not applied to legislation.gov.uk. Reg. 54(e) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(b))
- Annex 1 Pt. 7 para. 1 substituted by S.I. 2019/705, reg. 54(e)(iv) (as substituted) by S.I. 2020/1504 reg. 17(10)(b)
- Annex 1 Pt. 1 para. 2 words substituted by S.I. 2019/705 reg. 54(a)(i) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 1 para. 4(c) words substituted by S.I. 2019/705 reg. 54(a)(ii) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 1 para. 8 words substituted by S.I. 2019/705 reg. 54(a)(iii) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 2 para. 1.1(b) words substituted by S.I. 2019/705 reg. 54(b)(i) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 2 para. 1.1(e)(v) words substituted by S.I. 2019/705 reg. 54(b)(ii) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 2 para. 1.1(e)(vi) words substituted by S.I. 2019/705 reg. 54(b)(iii) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 2 para. 7 words substituted by S.I. 2019/705 reg. 54(b)(iv) (This amendment not applied to legislation.gov.uk. Reg. 54(a)(b) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(a))
- Annex 1 Pt. 7 para. 1.8(h) words substituted by S.I. 2019/705 reg. 54(e)(iv) (This amendment not applied to legislation.gov.uk. Reg. 54(e) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(10)(b))
- Annex 1 Pt. 1 para. 2 words substituted by S.I. 2019/705, reg. 54(a)(i) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 1 para. 4 words substituted by S.I. 2019/705, reg. 54(a)(ii) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 1 para. 8 words substituted by S.I. 2019/705, reg. 54(a)(iii) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 2 para. 1 words substituted by S.I. 2019/705, reg. 54(b)(i) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 2 para. 1 words substituted by S.I. 2019/705, reg. 54(b)(ii) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 2 para. 1 words substituted by S.I. 2019/705, reg. 54(b)(iii) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)
- Annex 1 Pt. 2 para. 7 words substituted by S.I. 2019/705, reg. 54(b)(iii) (as substituted) by S.I. 2020/1504 reg. 17(10)(a)

- Annex 1 Pt. 7 para. 1 words substituted by S.I. 2019/705, reg. 54(e)(iii) (as substituted) by S.I. 2020/1504 reg. 17(10)(b)
- Annex 3 para. 1 words omitted by S.I. 2019/705 reg. 56(a)(ii)
- Annex 3 point C para. 3.1(4) words omitted by S.I. 2019/705 reg. 56(c)
- Annex 3 para. 1(3) words substituted by S.I. 2019/705 reg. 56(a)(i)
- Annex 2 s. 2para. 1.3.1 words omitted by S.I. 2019/705 reg. 55(a)(ii)
- Annex 2 s. 2para. 2 words omitted by S.I. 2019/705 reg. 55(a)(ix)(aa)
- Annex 2 s. 2para. 1.1.2(b) words substituted by S.I. 2019/705 reg. 55(a)(i)
- Annex 2 s. 2para. 1.3.2.1 words substituted by S.I. 2019/705 reg. 55(a)(iii)
- Annex 2 s. 2para. 1.3.2.2 words substituted by S.I. 2019/705 reg. 55(a)(iv)
- Annex 2 s. 2para. 2 words substituted by S.I. 2019/705 reg. 55(a)(ix)(bb)
- Annex 2 s. 2para. 1.4.2 words substituted by S.I. 2019/705 reg. 55(a)(v)
- Annex 2 s. 2para. 1.4.4.1 words substituted by S.I. 2019/705 reg. 55(a)(vi)
- Annex 2 s. 2para. 1.5 words substituted by S.I. 2019/705 reg. 55(a)(vii)
- Annex 2 s. 2para. 1.6.4 words substituted by S.I. 2019/705 reg. 55(a)(viii)
- Art. 4(1)(b) words substituted by S.I. 2019/705 reg. 49 (This amendment not applied to legislation.gov.uk. Reg. 49 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 17(9))
- Art. 4(1)(b) words substituted by S.I. 2019/705, reg. 49 (as substituted) by S.I. 2020/1504 reg. 17(9)