ANNEX I

METHOD VALIDATION

1. INTRODUCTION

A. For the purpose of implementing Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003, this Annex provides 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. All guidance documents referred to in this Annex or produced by the Community Reference Laboratory (CRL) shall be made available by the CRL.

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 technical provisions and this in order to certify that the method validated is fit for the purpose of enforcement of Regulation (EC) No 1829/2003.

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.

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.

E. The validation process will be carried out by the CRL according to internationally accepted technical provisions.

F. The CRL, together with ENGL, shall provide further information about the operational procedures of the validation process and shall make the documents available.

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.

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.

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.

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.

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.

3. The method shall be developed taking the following documents in consideration as appropriate:
   - General requirements and definitions: draft European standard prEN ISO 24276:2002,
   - Nucleic acid extraction prEN ISO 21571:2002,
   - Quantitative nucleic acid based methods: draft European standard prEN ISO 21570:2002,
   - Protein based methods: adopted European standard EN ISO 21572:2002,
   - Qualitative nucleic acid based methods: draft European standard prEN ISO 21569:2002.

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.

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. 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.,
   - 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.

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 technical provisions about the appropriate formats for these data.

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.

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.

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 technical provisions used in the testing.

4. SAMPLES OF THE FOOD AND FEED AND THEIR CONTROL SAMPLES

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 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 CRL for the specific application for authorisation.

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EC) No 641/2004. 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 1 point 1.A word omitted by S.I. 2019/705 reg. 46(a)
- Annex 1 point 2.B word substituted by S.I. 2019/705 reg. 46(f)
- Annex 1 point 3.A word substituted by S.I. 2019/705 reg. 46(g)(i)
- Annex 1 word substituted by S.I. 2019/705 reg. 46(h)
- Annex 1 point 1.C words omitted by S.I. 2019/705 reg. 46(c)
- Annex 1 point 1.F words omitted by S.I. 2019/705 reg. 46(d)
- Annex 1 point 1.G words omitted by S.I. 2019/705 reg. 46(e)
- Annex 1 point 3.A words omitted by S.I. 2019/705 reg. 46(g)(ii)
- Annex 1 point 1.B words substituted by S.I. 2019/705 reg. 46(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. 45
- Art. 3(1)(a) words omitted by S.I. 2019/705 reg. 44(a)
- Art. 3(1)(d) words omitted by S.I. 2019/705 reg. 44(b)