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Foodstuffs — Nucleic acid based methods of analysis of genetically modified organisms and derived products — Information to be supplied and procedure for the addition of methods to ISO 21569, ISO 21570 or ISO 21571

Produits alimentaires — Méthodes basées sur les acides nucléiques pour l'analyse des organismes génétiquement modifiés et des produits dérivés — Informations à fournir et procédure pour l'addition de méthodes à l'ISO 21569, l'ISO 21570 ou l'ISO 21571

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 21098 was prepared by Technical Committee ISO/TC 34, *Food products*.

The reasons why this document is published as a Technical Specification are given in the Introduction.

Introduction

ISO has an obligation to ensure that the international standards it develops, adopts and publishes are globally relevant. Among the criteria detailed in Annex 4, paragraph 10, of the Second Triennial review of the operation and implementation of the Barriers to Trade Agreement, dated 13 November 2000, it is stated that a globally relevant standard should be performance based as opposed to design prescriptive. Thus any method submitted for inclusion in an International Standard should contain sufficient information for its performance to be judged.

Although ISO 24276 states "*The criteria for the selection of methods are listed in the standards on the detection of genetically modified organisms and derived products, ISO 21568, ISO 21569, ISO 21570 and ISO 21571. Acceptable levels of performance for methods included in the annexes are those which have preferably been collaboratively trialed/single laboratory validated. Methods selected for inclusion in the annexes have either been validated according to ISO 5725, or the Harmonized Protocol (Horwitz 1995) or according to Thompson et al. (2002)*", there is insufficient guidance in these documents to allow the analyst to test whether a method is specifically suitable for inclusion in the annexes. It is important that an International Standard or Technical Specification should be performance based. For a standard to be performance based, a clear definition of performance characteristics must be available.

It was noted at the 5th meeting of ISO/TC 34/WG 7, held 18th to 20th February 2004 in Seoul, Korea, that there is no formal process for submitting methods for inclusion in the standards. Although a number of specific methods have been proposed as part of the proposed standards (ISO 21569, ISO 21570 and ISO 21571) and associated general document (ISO 24276), there is not sufficient clarity for submitters to be able to judge whether a method meets the standard, and no mechanism is in place to govern acceptability and/or adoption of such method or for retaining methods in the standards.

Therefore, this Technical Specification was developed in order to provide guidance and to define the performance characteristics that should be supplied for each method in order to ensure the global relevance of these standards, and to delineate the process for adding, amending and retaining methods annexed to the standards.

Foodstuffs — Nucleic acid based methods of analysis of genetically modified organisms and derived products — Information to be supplied and procedure for the addition of methods to ISO 21569, ISO 21570 or ISO 21571

1 Scope

This Technical Specification defines the principles and specifies the nature of the information to be supplied for acceptance of a method as an annex to ISO 21569, ISO 21570 or ISO 21571. It also specifies the process for adding, amending and retaining methods annexed to these standards. This Technical Specification is necessary in order to attain consistency in methods that are to be employed as part of the standards. It does not cover the specifics of the development of a method or laboratory set-up. The operation of laboratories is covered in ISO/IEC 17025.

Method validation is instrumental in assessing the reliability of a test method. Its central role is to establish numerical values for the performance criteria that are to be established. ISO 24276 includes details on method validation, taking into consideration specific technical issues related to the detection of genetically modified organisms and derived products. Given the attention to, and widespread use of deoxyribonucleic acid-based tests or protein-based tests, and the implications to trade of any discrepancies in test results, a single-laboratory validation is most likely not warranted in this case and a multi-centre method validation could be performed according to the international guidelines.

2 Deoxyribonucleic acid (DNA)-based analysis

DNA-based analysis is commonly performed using polymerase chain reaction (PCR), although ISO 21569, ISO 21570 and ISO 21571 also allow for other methods. DNA is a high-molecular weight polymer that may be degraded during food processing by, for example, heat, enzymes and mechanical shearing. In addition, the DNA may be chemically altered by the formation of adducts, or by loss of the bases. Any degradation of the DNA shall be considered when assessing method validation and applying performance criteria. Degradation of DNA will affect the limit of detection and the limit of quantitation of the tests. It is important that the performance criteria for a method consider this effect. Additionally, it is important to point out the restrictions that method(s) may have in certain food matrices.

The annexes in ISO 21569, ISO 21570 and ISO 21571 should contain information on performance criteria from which methods fit for ISO purposes may be selected. It is possible that two different methods for the same event/sequence, both fulfilling the performance criteria, once established, will be included in the annexes.

It should be noted that, due to the limitations of the instruments and other factors, quantitative PCR methods in general do not follow a Gaussian distribution for blank values around the zero. Thus, the determination of the limit of detection and limit of quantitation cannot be carried out assuming such a distribution, and will not follow the procedures outlined, for example, in ISO 11843-1 Thus detection limits for quantitative PCR methods cannot be determined using the mean and standard deviation of the blank samples, and shall be determined experimentally, or methods shall be performed at levels which are significantly above the detection limit.

3 Multi-laboratory studies

Under certain circumstances (i.e. when the conduct of a formal collaborative trial is not practicable), methods may be validated via single laboratory validation (see Reference [11]). The methods used for determination of the presence of material originating from biotechnology-derived crops and food are able to be, and are intended to be, performed at multiple laboratories and shall therefore be validated by multi-laboratory

collaborative studies. The results of such studies may be incorporated by reference to the relevant scientific publication(s), in which case copies of the publications shall be submitted to the expert group when submitting the method.

At the time that ISO 21569, ISO 21570 and ISO 21571 are being prepared, few methods have completed a full multi-laboratory validation on a multi-regional basis. Therefore, as an interim measure, methods may be appended as informative annexes to the standards, after a properly conducted single-laboratory validation, or after validation in a small number of laboratories, providing that all the other criteria have been met. Methods which have been validated on a limited basis but are lacking full interlaboratory validation data, may be temporarily endorsed for a period not exceeding 3 years. Such methods will be reviewed within 3 years, at which time they should have been properly validated in a full interlaboratory collaborative study.

4 Description of information to be supplied about a method submitted to be annexed to ISO 21569, ISO 21570 and ISO 21571

The proposed informative annex should contain information about the performance characteristics of a method. This includes specific information on the multi- or single-laboratory trial, including relevant information obtained during prevalidation of the method (e.g. variation of parameters, reagents).

The method should be in a format that conforms to the template and be validated according to internationally accepted norms (see Reference [12]). Templates are given in Annexes A, B and C. A complete and detailed description of all the components of the method shall be included. In particular, the description shall address the following.

a) *Scientific basis*: An overview of the principles of the method, such as DNA molecular biology based (e.g. for real-time PCR) information should be provided. References to relevant scientific publications are useful.

b) *Scope/Applicability*: The objective of the method and the relevance of the method should be indicated. The matrix is of particular concern for these methods. Thus the method shall in particular give an indication of the matrix (e.g. processed food, raw materials, DNA solution), the type of samples (e.g. seeds, flour, pizza, cookies) and the range to which the method can be applied (range of use). Relevant limitations of the method should also be addressed (e.g. interference by other analytes or inapplicability to certain situations). Limitations may include possible restrictions due to the costs, equipment or specific and non-specific risks implied for either the operator and/or the environment (see Reference [13]). The allelic and copy number stability of the target sequence should be considered for cultivars of different geographic and phylogenic origins as per ISO 21570.

c) *Selectivity:* Empirical results from testing the method with non-target transgenic events and non-transgenic plants should be provided where possible. This testing should include closely related events and cases were the limits of the selectivity are truly tested.

d) *Reference materials:* Reference materials are not readily available for many events. However, as with any method, their use is encouraged. When a reference material becomes available, method submitters are encouraged to test the methods against these materials and include the information by amendment to the standard.

e) *Analytical controls:* Controls shall be clearly specified and their interpretation recorded. These may include positive and negative controls, their detailed contents, the extent to which they should be used, and the interpretation of the results obtained.

f) *Trueness and precision:* Information on the trueness and precision of the method shall be supplied as far as is feasible. “Trueness” refers to the closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value. “Precision” refers to the closeness of agreement between test results (see ISO 5725-1 for details).

g) *Instrument specificity:* The required equipment for application of the method should be clearly described, with regards to the analysis *per se* and also to the sample preparation. An indication of costs, timing, practical difficulties, and of any other factor that could be of importance for the operators should also be indicated. Instrument specificity can also be critical to a method. It is however, the policy of ISO not to

specify to a particular instrument wherever possible. Therefore, method developers and submitters are encouraged to validate methods on a variety of instruments. They should indicate in the method the instrument(s) on which the method was validated.

h) *Practicability of the method:* information should also be given on the practicability of the method, where known, such as an estimate of the time and resources required to perform the method on a specific number of samples.

i) *Specification of the prediction model/mathematical model needed for the method:* If the derivation of the results relies upon a mathematical relationship, this shall be outlined and recorded (e.g. a regression line or calibration curve obtained by other means). Instructions for the correct application of the model should be provided. These should include, depending on the method, a recommended number and range of levels to be analysed, minimum number of replicates to be included or the means to evaluate the fitness for purpose, and criteria for setting instrument thresholds.

j) *Criteria for acceptance of data:* The criteria adopted to interpret results and to make inferences shall be described in full detail. This includes the handling of, for example, conflicting results from replicate samples.

k) *Sensitivity and range:* The sensitivity of a method is defined as its ability to determine small differences in analyte concentrations. Empirical results from testing the method at different concentrations shall be provided in order to demonstrate the range of use of the method [see b)].

l) *Robustness testing:* The method provider should describe empirical results from testing the method against small but deliberate variations in method parameters.

m) *Performance requirement:* If the performance of a particular component of the method (e.g. the amplitaq enzyme) is particularly critical to performance of a method, then information as to how an operator can independently test the performance of the component should be included. This information will be important for the in-house validation that is necessary upon adoption of a method in any particular laboratory.

n) *Intellectual property and related issues:* Any intellectual property and related issues should be indicated.

5 Validation of methods

5.1 General

The method performance study or method validation establishes the performance characteristic for a specific method application, i.e. a specific analytical procedure for a well-defined scope. For a detailed discussion and explanation of the descriptions, refer to the Procedural Manual of Codex Alimentarius (see Reference [14]) and pertinent International Standards (e.g. the ISO 5725 series). Some of the most pertinent terms are outlined in Clause 4.

A method should be validated using the conditions under which it will be performed. Thus, it should not be tested using an unreasonable number of amplification cycles. Most PCRs can be expected to result in analytical artefacts if operated with too many cycles and/or under non-optimal conditions. Validation can include limit of detection (LOD) and range of use and, if applicable, the limit of quantitation (LOQ) for DNA detection methods.

5.2 Validation of quantitative methods

The validation of methods is described in ISO 5725-2 and the IUPAC/ISO/AOAC harmonized protocol (see Reference [12]). It is worth noting that a determination of an LOD or LOQ is not necessarily needed to establish the validity of a method for a given application. For example, if the method is to be used for concentrations in grams per kilogram, it does not add much value if an LOD is determined to be 1 ng/kg. In this and similar cases, the reliability of the method will be proven by the other parameters. Similar considerations apply for the LOQ. However, it is always necessary to determine the range of use of the method in the validation study. The method will only be applicable in that range.

If it is desired to determine the LOD, it is common practice to estimate it as the value of the blank increased by three times the standard deviation of the blank. However, this approach relies on normal Gaussian distribution of the blank measurements around zero. Its use is not valid for many instrument-based methods such as quantitative PCR, in which the distribution of measurement values for blanks is typically truncated at zero and therefore not normally distributed. The LOD in these cases shall be experimentally determined unless the range of use is well above the LOD.

For a quantitative method, it is important to know if the LOQ is close to the values to be measured in a particular matrix. The traditional approach, in which the LOQ is estimated to be the blank increased by 6 to 10 times the standard deviation of the blank, is not valid for many instrument-based methods such as quantitative PCR.

It is important to note that a method should be validated using the conditions under which it will be performed. Thus, it should not be tested using an unreasonable number of amplification cycles. Most PCR methods can be expected to result in analytical artefacts if operated with too many cycles and/or under non-optimal conditions.

5.3 Validation of qualitative methods

The validation of qualitative methods has not been discussed extensively nor harmonized.

A qualitative PCR shall be validated in the same way as it is intended to be used. The sensitivity of the method shall be shown to be such that it can reliably detect one positive particle (e.g. a single grain) in a pool, and does not give rise to a significant number of false positives. A concept of using false-positive and false-negative rates to describe the accuracy and precision of a qualitative assay has been developed for microbial assays (see ISO 16140 and References [15] and [16]). This concept may be applied to qualitative PCR assays. A critical issue in the validation of this type of method is the availability of test materials that are known to be positive and negative.

By their very nature, qualitative tests result only in yes/no answers. The measures of precision and accuracy are the frequencies of false negative and/or false positive results. False negative results indicate the absence of a given analyte when in fact the analyte is present in the sample; false positive results indicate the presence of an analyte that is not present in the sample. An increase in false negative results will be observed when the amount of analyte approaches the LOD of the method. Like the LOD for quantitative methods, the LOD for a qualitative method may be defined as the concentration at which a positive sample yields a positive result at least 95 % of the time. This results in a rate of false negative results of 5 % or less. During validation of a qualitative PCR assay, it is also important to determine the number of false positive results (a positive result obtained using a sample that is known to be negative).

Both false positive and false negative results are expressed as rates.

5.3.1 False-positive rate

This is the probability that a known negative test sample has been classified as positive by the method. The false-positive rate, R_{fp}, is the number of misclassified known negatives, M_n, divided by the total number of negative test samples, N_n (misclassified positives plus the number of correctly classified known negatives), obtained with the method.

For convenience this rate can be expressed as percentage:

$$R_{fp} = \frac{M_n}{N_n} \times 100\ \%$$

5.3.2 False-negative rate

This is the probability that a known positive test sample has been classified as negative by the method. The false-negative rate, R_{fn}, is the number of misclassified known positives, M_p, divided by the total number of positive test samples, N_p (misclassified positives plus the number of correctly classified known positives), obtained with the method.

For convenience this rate can be expressed as percentage:

$$R_{\text{fn}} = \frac{M_{\text{p}}}{N_{\text{p}}} \times 100\ \%$$

In order to demonstrate the false-negative rate for a qualitative assay, a series of samples (e.g. grain pools) with a constant, known concentration of positive material in a pool of negative material (e.g. 1 positive kernel in 199 conventional maize kernels) shall be analysed and the results evaluated. It is important to note that the concept of confidence intervals and statistical uncertainty shall be applied to the risk of false positive and/or false negative results as well. The desired level of confidence determines the size and number of pools that need to be tested. For example, 100 positive test results obtained from 100 independent measurements on truly positive samples lead to the conclusion that the level of false-negative results is below 4,5 % at a confidence level of 99 % for the tested concentration of positive kernels (expressed as the number of positive kernels in a pool of negative kernels).

A method should be validated using the conditions under which it will be performed. Thus, it should not be tested using an unreasonable number of amplification cycles. Most PCRs can be expected to result in analytical artefacts if operated with too many cycles and/or under non-optimal conditions.

6 Process for adding, amending and retaining methods (as annexes)

6.1 Expert group for consideration of the methods

Within ISO/TC 34/WG 7 (or its successor) an ad-hoc expert group shall be formed, and shall convene (by electronic or other means) to consider any submitted methods, and shall report to the secretariat at least 6 weeks prior to the next WG 7 meeting. The report shall be circulated to the WG 7 members at that time.

The expert group shall be composed of at least 5 experts nominated by member bodies. The group shall have an international membership, with representation that is regionally balanced.

6.2 Addition of methods to the standards

Each method shall be included as a separate annex, so as to simplify the formatting and amendment of the documents.

Methods intended for inclusion in the annexes should be submitted by the member bodies to the secretariat for distribution to the expert group at least 12 weeks before the meeting of ISO/TC 34/WG 7. This would allow the expert group to receive and consider the methods, and for their report to be circulated to the participants before the meeting. The WG 7 shall consider the report of the working group at the following meeting.

Methods shall meet the requirements of this Technical Specification. The WG 7 shall consider the methods or method amendments at the meeting and endorse the method or amendment to be included, or shall refer the method back to the submitting member body for the supplying of any further information that is needed.

Methods which have been validated on a limited basis (such as single-laboratory validation) but are lacking full interlaboratory validation data, may be temporarily endorsed for a period not exceeding 3 years, at which time they shall be reviewed again by the expert group.

6.3 Amending of methods in the standards

Amendments to the methods should be submitted by the member bodies to the secretariat for distribution to the expert group at least 12 weeks before the meeting of ISO/TC 34/WG 7 as for new methods. This would allow the expert group to receive and consider the methods, and for their report to be circulated to the participants before the meeting.

6.4 Retention of methods in the standards

It is acknowledged that the standards in preparation already contain methods as informative annexes that have been submitted through the European Committee for Standardization (CEN). There is no intention to remove these methods from the standards. These methods shall be considered for retention by the relevant expert group prior to the meeting of ISO/TC 34/WG 7 that occurs before the decision on the systematic review of the given standard is due. It is expected that the method submitters and member bodies would use this time to ensure that the methods meet the requirements of this Technical Specification. Submission of suitable information prior to this time would allow the methods to be considered and confirmed at an earlier time point. Methods that are judged to meet the requirements of this Technical Specification shall be recommended for retention.

Annex A
(normative)

Template for supplying the required information about a method to be annexed to ISO 21569

A.1 Title

The title of the method should include an indication of the species and event or sequence to be detected.

A.2 Purpose, relevance and scientific basis

It is suggested that the name and address of the person or institution submitting the method be included.

A.3 Scope

Describe the species, transgenic event and matrix on which the method has been validated.

A.4 Validation status and performance criteria

A.4.1 Robustness of the method

Give information about the variations in reaction conditions that have been tested. Also list conditions which are known to interfere (in a positive or negative fashion) with the method.

A.4.2 Intralaboratory trial

Give information obtained from the intralaboratory trial, including the relative standard deviation of repeatability.

A.4.3 Collaborative trial

Give information obtained from the multi-laboratory trial, including the relative standard deviation of reproducibility.

A.4.4 Molecular selectivity

A.4.4.1 General

Describe the DNA sequence that is targeted by the method.

A.4.4.2 Experimental

Describe the results of experiments designed to test selectivity, e.g. lack of a positive result with non-transgenic material of the same and different species, and with transgenic material of the same and different species, which is expected to give a negative result.

A.4.4.3 Theoretical

Describe the result of the sequence homology search for DNA sequences in data bank searches (e.g. NCBI, EMBL) and the type of search performed (include the date).

A.5 Principle and summary

Give a general description of the PCR or other process that forms the basis of the analytical method.

A.6 Definitions

See ISO 5725-1 and ISO 24276 for details.

A.7 Sample type and amounts

Give details of the type of laboratory and test samples required (analyte and matrix), with particular reference to issues of sampling.

A.8 Limit of detection (LOD) and range of use

The detection limit and range of application of the method should be given in terms of percent (mass fraction), and/or in terms of copies of DNA, together with the methods used to determine these values. These limits are typically expressed at a 95 % confidence level. The values should be established via an interlaboratory trial using appropriate reference samples. Information may also derive from intralaboratory studies as a temporary measure, but the method will be examined within the next 3 years to determine whether the method is to be retained in the standard.

A.9 Estimation of measurement uncertainty

Uncertainty arises from many sources, including the size of the laboratory sample, sampling of the test sample from the laboratory sample, measurement of the DNA concentration in the extracts, and the sampling of the DNA into the reactions, as well as the analytical variation. An estimate of the measurement uncertainty may be derived from intra/interlaboratory study, or from estimates of the components as described in Reference [17].

A.10 Interferences

List the conditions or materials that are known to interfere (in a positive or negative fashion) with the method, such as matrices that yield samples that interfere with the PCR method. Also list any known information about interferences from mixtures of the matrix with other matrices.

A.11 Physical/Environmental conditions

Specify the conditions (if there are special environmental conditions for performing the analysis), such as normal laboratory conditions, or use of a cold room, performance of certain steps at specific temperatures etc. (except, for example, cycling conditions of PCR).

A.12 Apparatus/Equipment

List specific equipment, including thermocycler, blender or other grinding apparatus, sieves, apparatus or equipment for measuring the DNA concentration, but not general laboratory disposables and equipment.

A.13 Reagents/Supplies

List any specialized reagents. For the quality of reagents and for specific reagents used, see ISO 24276.

A.14 Sample collection, transport, preservation and storage

Describe any provisions regarding sample collection and sampling, as well as storage conditions.

A.15 Test sample preparation

Give details of the test sample preparation, such as grinding and sieving steps (e.g. a reference to a method described in ISO 21571 if the method has been validated using DNA isolated by that method).

A.16 Instrument calibration

Instruments (e.g. thermocyclers) should be calibrated as per ISO/IEC 17025.

A.17 Analysis steps

Give a general description of the analysis steps, which will be described in detail in this section.

A.17.1 Preparation of the DNA extracts

This may be a reference to a method described in ISO 21571 if the detection method has been validated using DNA isolated by that method.

A.17.1.1 DNA extraction

Describe the method used to extract DNA from this matrix.

A.17.1.2 DNA quantitation

Give the method used to determine the amount of DNA extracted from the test sample.

A.17.1.3 DNA integrity evaluation

Give the method used to determine the integrity of DNA obtained from the extractions.

A.17.2 PCR reagents

A.17.2.1 Thermostable DNA-polymerase, buffers, etc.

Give a description of the required characteristics of these reagents.

A.17.2.2 Primers

Describe the sequences of the primers and probes, and the preparation and/or source of the primers, as well as other components such as TaqMan® or other probes.

To avoid confusion between bases, a lower-case 'g' shall be used to clearly differentiate between 'G' and 'C' in the description (i.e. C g A and T shall be used to indicate bases).

A.17.3 Procedure

Describe the procedure in sufficient detail that a trained analyst can perform the method, including any special steps involved, and the preparation of the master mix where applicable, as well as the amount of DNA (test portion) that is to added to the reaction.

A.17.3.1 PCR controls

List both the positive and negative controls that are necessary, as required in ISO 24276.

A.17.3.2 Preparation of standards

Describe the preparation of standards for the PCR step and any other standards (such as size standards) required.

A.17.3.3 Temperature-time programme (PCR)

Describe the temperature-time programme used and the type of machine used to supply these cycles (e.g. block cyclers).

A.17.3.4 Accept/Reject criteria

A validated method includes criteria from which an observed measurement result can be accepted as valid. Describe accept/reject criteria for the analysis.

A.17.3.5 Identification

If the result of the analysis can be verified, indicate how this can be achieved.

A.18 Sample identification

All samples shall be identified unambiguously.

A.19 Calculations

Describe any calculations or models used to derive the analytical result.

A.20 Record keeping

Record keeping should conform to ISO/IEC 17025.

A.21 Reporting

Reporting should be carried out as specified in ISO 24276 and other applicable standards (e.g. ISO/IEC 17025).

A.22 Safety measures

Describe any particular safety measures (not including country/region specific issues) that should be brought to the attention of the analyst.

A.23 Pollution prevention/Waste disposal

Appropriate local best practices should be adhered to.

A.24 Appendices (Tables, Diagrams, etc.)

The method should include such diagrams and tables as needed to inform the analyst. The method should not contain any appendices, as they are themselves annexes.

Annex B
(normative)

Template for supplying the required information about a method to be annexed to ISO 21570

B.1 Title

The title of the method should include an indication of the species and event or sequence(s) to be detected.

B.2 Purpose, relevance and scientific basis

It is suggested that the name and address of the person or institution submitting the method be included.

B.3 Scope

Describe the species, transgenic event, and matrix on which the method has been validated. If a method depends on the comparison of copy numbers of genes determined by two measurements (e.g. comparison of target and taxon-specific gene), information, including validation data, shall be supplied for each component.

B.4 Validation status and performance criteria

B.4.1 Robustness of the method

Give information about the variations in reaction conditions that have been tested. Also list conditions that are known to interfere (in a positive or negative fashion) with the method.

B.4.2 Intralaboratory trial

Give information obtained from the intralaboratory trial, including the relative standard deviation of repeatability.

B.4.3 Collaborative trial

Give information obtained from the multi-laboratory trial, including the relative standard deviation of reproducibility.

B.4.4 Molecular selectivity

B.4.4.1 General

Describe the DNA sequence that is targeted by the method.

B.4.4.2 Experimental

Describe the results of experiments designed to test selectivity for each component, i.e. lack of a positive result with non-transgenic material of the same and different species, and with transgenic material of the same and different species, which is expected to give a negative result.

B.4.4.3 Theoretical

Describe the result for each component of the sequence homology search for DNA sequences in data bank searches (e.g. NCBI, EMBL) and the type of search performed (include the date).

B.5 Principle and summary

Give a general description of the PCR or other process, which forms the basis of the analytical method(s).

B.6 Definitions

See ISO 5725-1 and ISO 24276 for details.

B.7 Sample type and amounts

Give details of the type of laboratory and test samples required (analyte and matrix), with particular reference to issues of sampling.

B.8 Limit of detection (LOD), limit of quantitation (LOQ) and range of use

The detection limit, limit of quantitation, and range of application of the method for each component should be given in terms of percent (mass fraction), and may also include information on the detection limit in terms of copies of DNA, together with the methods used to determine these values. These limits are typically expressed at a 95 % confidence level. The values should be established via an interlaboratory trial using appropriate reference samples. Information may also derive from intralaboratory studies as a temporary measure, but the method(s) will be examined within the next 3 years to determine whether the method is to be retained in the standard.

B.9 Estimation of measurement uncertainty

Uncertainty arises from many sources, including the size of the laboratory sample, sampling of the test sample from the laboratory sample, measurement of the DNA concentration in the extracts, and the sampling of the DNA into the reactions, as well as the analytical variation. An estimate of the measurement uncertainty for each component may be derived from intra-/interlaboratory studies, or from estimates of the components as described in Reference [16].

B.10 Interferences

List conditions or materials which are known to interfere (in a positive or negative fashion) with the method(s), such as matrices that yield samples that interfere with the PCR method. Also list any known information about interferences from mixtures of the matrix with other matrices.

B.11 Physical/Environmental conditions

Specify conditions (if there are special environmental conditions for performing the analysis), such as normal laboratory conditions, or use of a cold room, performance of certain steps at specific temperatures (except, for example, cycling conditions of PCR).

B.12 Apparatus/Equipment

List specific equipment, including thermocycler, blender or other grinding apparatus, sieves, apparatus or equipment for measuring DNA concentration, but not general laboratory disposables and equipment.

B.13 Reagents/Supplies

List any specialized reagents. For quality of reagents and for specific reagents used, see ISO 24276.

B.14 Sample collection, transport, preservation and storage

Describe any provisions regarding sample collection and sampling, as well as storage conditions.

B.15 Test sample preparation

Give details of the test sample preparation, such as grinding and sieving steps (e.g. a reference to a method described in ISO 21571 if the method has been validated using DNA isolated by that method).

B.16 Instrument calibration

Instruments (e.g. thermocyclers) should be calibrated as per ISO/IEC 17025.

B.17 Analysis steps

Give a general description of the analysis steps, which will be described in detail in this section.

B.17.1 Preparation of the DNA extracts

This may be a reference to a method described in ISO 21571 if the detection method has been validated using DNA isolated by that method.

B.17.1.1 DNA extraction

Describe the method used to extract DNA from this matrix.

B.17.1.2 DNA quantitation

Give the method used to determine the amount of DNA extracted from the test sample.

B.17.1.3 DNA integrity evaluation

Give the method used to determine the integrity of DNA obtained from the extractions.

B.17.2 PCR reagents

B.17.2.1 Thermostable DNA-polymerase, buffers, etc.

Give a description of the required characteristics of these reagents.

B.17.2.2 Primers

Describe the sequences of the primers and the preparation and/or source of the primers, as well as other components, such as TaqMan® or other probes.

To avoid confusion between bases, a lower-case 'g' shall be used to clearly differentiate between 'G' and 'C' in the description (i.e. C g A and T shall be used to indicate bases).

B.17.3 Procedure

Describe the procedure in sufficient detail that a trained analyst can perform the method, including any special steps involved, and the preparation of the master mix where applicable, as well as the amount of DNA (test portion) that is to added to the reaction.

B.17.3.1 PCR controls

List both the positive and negative controls that are necessary, as required in ISO 24276.

B.17.3.2 Preparation of standards

Describe the preparation of standards for the PCR step and any other standards (such as size standards) required.

B.17.3.3 Temperature-time programme (PCR)

Describe the temperature-time programme used and the type of machine used to supply these cycles (e.g. block cyclers).

B.17.3.4 Accept/Reject criteria

A validated method includes criteria from which an observed measurement result can be accepted as valid. Describe accept/reject criteria for the analysis.

B.17.3.5 Identification

If the result of the analysis can be verified, indicate how this can be achieved.

B.18 Sample identification

All samples shall be identified unambiguously.

B.19 Calculations

Describe any calculations or models used to derive the analytical result. This includes criteria for choice of instrument settings (e.g. setting the threshold) and any manipulation of the data that is to be performed (e.g. comparison of copy numbers).

B.20 Record keeping

Record keeping should conform to ISO/IEC 17025.

B.21 Reporting

Reporting should be carried out as specified in ISO 24276 and other applicable standards (e.g. ISO/IEC 17025.

B.22 Safety measures

Describe any particular safety measures (not including country/region specific issues) that should be brought to the attention of the analyst.

B.23 Pollution prevention/Waste disposal

Appropriate local best practices should be adhered to.

B.24 Appendices (Tables, Diagrams, etc.)

The method should include such diagrams and tables as needed to inform the analyst. The method should not contain any appendices, as they are themselves annexes.