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Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions

Produits alimentaires — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés — Exigences générales et définitions

Reference number
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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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.

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 24276 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

The purpose of such an analysis is to identify and quantify genetic elements or proteins common to genetically modified organisms (GMOs) and their derived products in a given matrix.

The main focus of this International Standard is polymerase chain reaction (PCR) based methodologies. However, because of the rapid rate of technological change in this area, other technologies may be considered in the future.

The search for ingredients of genetically modified origin is performed by means of the following successive (or simultaneous) steps. After sample collection, nucleic acids or proteins are extracted from the test portion. Extracted analytes can be further purified, simultaneously or after the extraction process. Afterwards, they are quantified (if necessary), diluted (if necessary) and subjected to analytical procedures, such as PCR or Enzyme-Linked Immunosorbent Assay (ELISA). These steps are detailed in this International Standard and in the following documents:

EN/TS 21568, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling strategies*

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

ISO 21572, *Foodstuffs — Methods for the detection of genetically modified organisms and derived products — Protein based methods*

Specific information pertaining to protein detection methods is found in ISO 21572.

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions

1 Scope

This International Standard specifies how to use the standards for sampling strategies (EN/TS 21568), nucleic acid extraction (ISO 21571), qualitative nucleic acid analysis (ISO 21569), quantitative nucleic acid analysis (ISO 21570) and protein-based methods (ISO 21572), and explains their relationship in the analysis of genetically modified organisms in foodstuffs.

It contains general definitions, requirements and guidelines for laboratory set-up, method validation requirements, description of methods and test reports.

It has been established for food matrices, but could also be applied to other matrices (e.g. seeds, feed and plant samples from the environment).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

3 Terms and definitions

For the purpose of this document, the terms and definitions given in ISO 5725-1 concerning validation, those in Reference [1] and the following apply.

3.1 General definitions

3.1.1
target taxon
taxon to which the genetically modified organism belongs

NOTE In this context, taxon usually means species but it could be of lower or higher taxonomic rank.

3.1.2
laboratory sample
sample as prepared for sending to the laboratory and intended for inspection or testing

[ISO 7002:1986]

3.1.3
test sample
test portion
sample, as prepared for testing or analysis, the whole quantity being used for analyte extraction at one time

3.1.4
specificity
property of a method to respond exclusively to the characteristic or analyte under investigation

3.1.5
sensitivity
change in the response divided by the corresponding change in the concentration of a standard (calibration) curve

NOTE This is the slope of the analytical calibration curve.

3.1.6
limit of detection
LOD
minimum amount or concentration of the analyte in a test sample which can be detected reliably but not necessarily quantified, as demonstrated by a collaborative trial or other appropriate validation

NOTE See Reference [2] for collaborative trial and Reference [3] for validation.

3.1.7
limit of quantitation
LOQ
〈analytical procedure〉 lowest concentration or amount of the analyte in a test sample which can be quantitatively determined with an acceptable level of precision and accuracy, as demonstrated by a collaborative trial or other appropriate validation

NOTE See Reference [2] for collaborative trial and Reference [3] for validation.

3.1.8
accuracy
closeness of agreement between a test result and the accepted reference value

3.1.9
trueness
closeness of agreement between the average value obtained from a large series of test results and an accepted reference value

NOTE The measure of trueness is usually expressed in terms of bias. Trueness has been referred to as “accuracy of the mean”.

3.1.10
precision
closeness of agreement between independent test results obtained under stipulated conditions

NOTE 1 Precision depends only on the distribution of random errors and does not relate to the true value or to the specified value.

NOTE 2 The measure of precision usually is expressed in terms of imprecision and computed as a standard deviation of the test results. Lower precision is reflected by a larger standard deviation.

NOTE 3 “Independent test results” means results obtained in a manner not influenced by any previous result on the same or similar test object. Quantitative measures of precision depend critically on the stipulated conditions. Repeatability and reproducibility conditions are particular sets of extreme conditions.

3.1.11
repeatability
precision under repeatability conditions

3.1.12
reproducibility
precision under reproducibility conditions

3.1.13
repeatability conditions
conditions where independent 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

3.1.14
reproducibility conditions
conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment

NOTE When different methods give test results that do not differ significantly, or when different methods are permitted by the design of the experiment (as in a proficiency study or a material-certification study for the establishment of a consensus value of a reference material), the term “reproducibility” may be applied to the resulting parameters. The conditions must be explicitly stated.

3.1.15
repeatability standard deviation
standard deviation of test results obtained under repeatability conditions

NOTE Repeatability standard deviation is a measure of the dispersion of the distribution of test results under repeatability conditions. Similarly “repeatability variance” and “repeatability coefficient of variation” could be defined and used as measures of the dispersion of test results under repeatability conditions.

3.1.16
reproducibility standard deviation
the standard deviation of test results obtained under reproducibility conditions

NOTE Reproducibility standard deviation is a measure of the dispersion of the distribution of test results under repeatability conditions. Similarly “reproducibility variance” and “reproducibility coefficient of variation” could be defined and used as measures of the dispersion of test results under reproducibility conditions.

3.1.17
repeatability limit
value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95 %

NOTE 1 The symbol used is r.

NOTE 2 When examining two single test results obtained under repeatability conditions, the comparison should be made with the repeatability limit $r = 2{,}8\ s_r$.

3.1.18
reproducibility limit
value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95 %

NOTE 1 The symbol used is R.

NOTE 2 When examining two single test results obtained under reproducibility conditions, the comparison should be made with the reproducibility limit $R = 2{,}8\ s_R$.

3.1.19
collaborative trial
interlaboratory study
study in which several laboratories detect and/or determine an analyte in one or more "identical" portions of homogeneous, stable materials under documented conditions

NOTE Guidelines for performing collaborative trials are elaborated in ISO 5725-2 and ISO/AOAC/IUPAC harmonized protocol [6].

3.1.20
fitness for purpose
applicability
scope of application of the method which identifies the matrix, analyte or species being measured, its concentration range and the type of study/monitoring effort for which the procedure, as judged from its performance characteristics, is suited

NOTE It also describes the known limitations of the method. [3]

3.1.21
practicability
ease of operations, in terms of sample throughput and costs, to achieve the required performance criteria and thereby meet the specified purpose

3.1.22
applicability range
range of quantitation/linearity/dynamic range
quantity interval within which the analytical procedure has been demonstrated by a collaborative trial or other appropriate validation to have a suitable level of precision and accuracy

NOTE See Reference [2] for collaborative trial and Reference [3] for validation.

3.1.23
measurement uncertainty
parameter associated with the result of a measurement, which characterizes the dispersion of the values that could reasonably be attributed to the analyte

3.1.24
screening method
method that will rapidly and reliably eliminate (screen) a large number of negative (or positive) test samples and restrict the number of test samples requiring the application of a rigorous method

NOTE 1 See Reference [4].

NOTE 2 In this International Standard, a screening method is a method to detect gene products (such as proteins) and/or genetic elements common to several GMOs (such as promoters, terminators, or other genetic elements of interest).

3.1.25
construct-specific method
method that targets a combination of inserted DNA sequences that are only found in GMO-derived material

3.1.26
event-specific method
method that detects a specific sequence that is only present in that event

NOTE This is commonly targeted at the integration-border region.

3.2 Terms relative to extraction and purification of DNA

3.2.1
DNA extraction
separation of DNA from the other components in a test sample

3.2.2
DNA purification
method resulting in a more purified DNA

NOTE In this context, purity refers to the reduction of observable and measurable effects of PCR inhibitors.

3.2.3
PCR quality DNA
DNA template of sufficient length, chemical purity and structural integrity to be amplified by PCR.

3.3 Terms referring to DNA amplification and PCR

3.3.1
identification of nucleic acid sequences
identity of nucleic acid sequences
establishment of identity by comparison with a reference nucleic acid fragment/sequence

NOTE For example, specific hybridization with a probe, matching restriction digest profiles or matching nucleic acid sequences.

3.3.2
junction region
DNA sequence encompassing two consecutive sequence elements, such as a promoter and the coding region of a gene

3.3.3
integration-border region
junction region where one element originates from the host organism and the other originates from the DNA introduced during transformation

3.3.4
taxon-specific (endogenous) target sequence
sequence known to be specific for the target taxon

NOTE 1 That is consistently present in the target taxon and absent in other taxa.

NOTE 2 There are at least two types of target taxon-specific sequences:

— variable number or multicopy sequences that can be used, for example, to assess the presence of nucleic acid from the target taxon;

— low copy number or single copy sequences that can also be used, for example, as a reference sequence to establish the background of target taxon genome equivalents in a quantitative analysis.

3.3.5
forward flow
principle of material/sample handling applied to ensure that the laboratory sample, raw and processed test portion (including amplified DNA) remain physically segregated during the whole procedure

3.4 Definitions referring to DNA and PCR controls

NOTE Controls applicable to protein-based methods are described in ISO 21572. The following definitions apply to DNA-based methods.

3.4.1
positive DNA target control
reference DNA, or DNA extracted from a certified reference material, or known positive sample representative of the sequence or organism under study

NOTE This control is used to demonstrate that the PCR reagents are working as intended.

3.4.2
negative DNA target control
reference DNA, or DNA extracted from a certified reference material, or known negative sample not containing the sequence under study

NOTE This control demonstrates that the results of analyses of test samples not containing the target sequence will be negative.

3.4.3
PCR inhibition control
reaction mixture that provides the means to monitor PCR inhibition of the assay for the specific sample of the target analyte

NOTE 1 This control allows the determination of the presence of soluble PCR inhibitors, which is particularly necessary in the case of negative amplification and of quantitative PCR.

NOTE 2 Generally, a known amount of target DNA is added to the reaction that is to be controlled. This could be the original target or a spike, for example a slightly modified target such as a competitor plasmid.

3.4.4
PCR reagent control
control containing all the amplification reagents except the extracted test sample template DNA

NOTE This control is used to demonstrate the absence of contaminating nucleic acids in the reagents. Instead of the template DNA, for example, a corresponding volume of nucleic acid free water is added to the reaction.

3.4.5
extraction blank control
control generated by performing all steps of the extraction procedure except the addition of the test portion

NOTE 1 For example by substitution of water for the test portion.

NOTE 2 This control is used to demonstrate the absence of contaminating nucleic acid during extraction.

3.4.6
positive extraction control
control used to demonstrate that the DNA extraction procedure has been performed in a way that will allow for extraction of a target DNA

NOTE For example by using a sample material known to contain the target DNA.

3.4.7
environment control
control used to determine that there is no nucleic acid contamination from, for example, the air in the laboratory

NOTE The control is a tube containing a suitable volume of nucleic acid free water that is left open to the air throughout the entire process.

3.5 Terms relative to reference materials

3.5.1
reference material
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]

3.5.2
certified reference material
reference material accompanied by a certificate, one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body

[ISO Guide 30]

3.6 Terms relative to quantitation

NOTE Controls applicable to protein-based methods are described in ISO 21572. The following definitions apply to DNA-based methods.

3.6.1
endogenous DNA sequence
defined reference DNA sequence native to the corresponding taxon

NOTE The endogenous DNA sequence can be used to determine the quantity of genome equivalents of the target taxon if the sequence is present in a constant copy number and does not show allelic variation among cultivars of the target taxon.

3.7 Terms relative to GMOs

3.7.1
GMO content
identity and quantity of GMO or GMO-derived material in the product

NOTE Generally, the GMO content is estimated by analyte detection (identification and quantitation).

4 Application to the relevant International Standards

4.1 General

Methods which are included in the annexes of ISO 21569, ISO 21570, ISO 21571 and ISO 21572 have been validated by collaborative trials[2], [8] or other appropriate validations[6]. The results of the validation and the method performance characteristics are described in each method.

These International Standards contain a number of individual methods deemed suitable for the detection of GMO-derived materials in foodstuffs. The specific choice of method(s) depends on the fitness for purpose, and the user of these standards is referred to the scope of each annex for further details.

The standards for detection of genetically modified organisms and derived products in foodstuffs are given in the Introduction. The inter-relationships of these International Standards are described in the flow diagram in Figure 1.

NOTE ISO/TS 21098 outlines requirements for methods to be annexed to ISO 21569, ISO 21570 and ISO 21571.

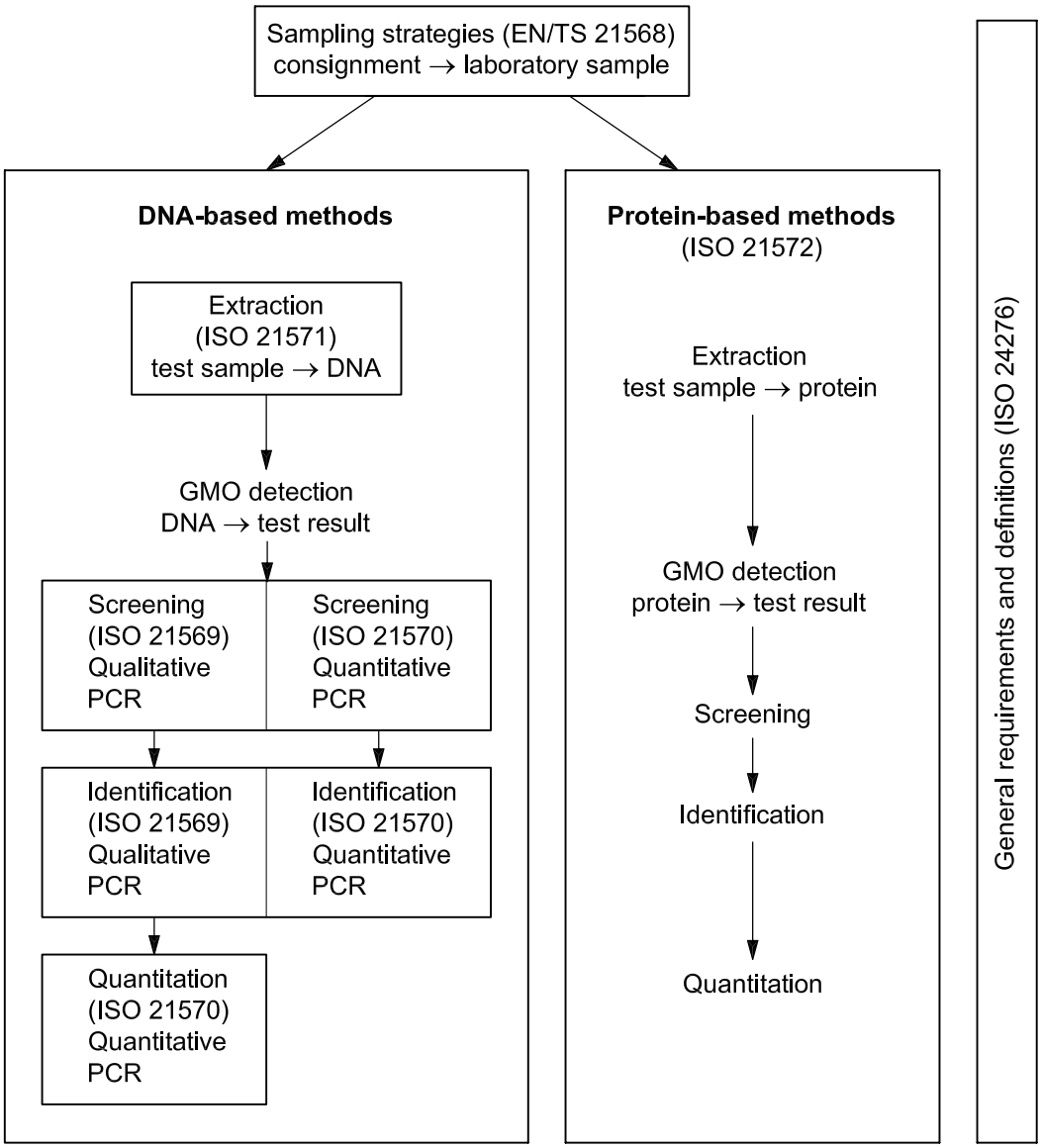

Figure 1 — Flow diagram of inter-relationships between the related standards

4.2 Guidance for the user on the selection of methods

4.2.1 General

The specificity of particular target analytes and detection methods may vary considerably. It is therefore important to ensure that the chosen method(s) provide the desired specificity. The guidance given in 4.2.2 and 4.2.3 may be useful.

4.2.2 Methods using protein as the analytical target

Proteins may be detected by the application of particular antibodies. In this case, a particular antibody is usually produced to detect a single protein. The degree of affinity of the antibodies for the protein will depend on the protein conformation after extraction. Specificity of the antibody used needs to be demonstrated (e.g. no cross-reactivity). Event-specific GMO detection by protein quantitation cannot be performed accurately if more than one GMO event expressing the same protein exists.

Screening methods may be useful to assess whether or not a product is likely to contain GMO-derived material based on the presence of the expressed protein. Examples of screening methods are lateral flow/dip stick format and qualitative ELISAs.

Quantitation of the GMO content in certain matrices can be performed by protein-based methods such as ELISA. The method shall be validated for the matrix to be analysed and standards shall be available for establishing a standard curve from which calculation of the protein content in test samples is performed.

4.2.3 Methods using DNA as the analytical target

The specificity of analytical methods using DNA as the target to determine the presence of GMO-derived material depends on the specific properties of the targeted DNA-sequence. The different applications and classifications of specificity are as follows.

a) Taxon-specific methods target DNA sequences found in a single taxon, usually a species but possibly of lower or higher taxonomic rank. The taxon-specific methods may be useful to assess the presence, quality and quantity of DNA derived from the taxon, and are sometimes used as the point of reference for relative quantitation of the GMO-derived material. Specificity needs to be established by experimental data.

b) Screening methods target DNA sequences found in several but not necessarily all transformation events. These sequences may also be found in non-GM material, for example due to the presence of natural viruses or bacteria. Screening methods may be useful to assess whether or not a product is likely to contain GMO-derived material. An example of a screening method is a qualitative PCR targeting the CaMV 35S promoter.

c) Construct-specific methods target DNA sequences that are only found in GMO-derived material, i.e. genetically engineered combinations of DNA sequence elements. Detection of a particular gene construct may be sufficient to identify the transformation event from which the DNA is derived, but the same gene construct may have been used in more than one transformation event, and the inserted number of copies of complete and/or truncated gene constructs per haploid GM-genome may vary between events.

d) Event-specific methods target DNA sequences that are only found in material derived from a single transformation event, usually a DNA sequence spanning the junction between the inserted DNA and the host genome (the integration border region). The number of copies of the event-specific DNA sequence is always one per haploid GM-genome. The event-specific methods are particularly suitable for identification of a specific event and, when used in a validated real-time PCR method, may also quantify the amount of DNA derived from a particular GMO.

4.3 Performance characteristics

4.3.1 General

Acceptable performance characteristics and levels of methods are those of the methods given in annexes of the relevant International Standards.

4.3.2 Limit of detection (LOD)

The LOD values for each analytical method as specified in the annexes of ISO 21569, ISO 21570 and ISO 21572 are based on data from collaborative trial validation and/or claims from the method developer, and are included for information purposes only. The LOD values reported from collaborative trial data generally refer to the lowest level of analyte that was observed to have a false negative rate of $\leqslant$ 5 % with a relative standard deviation of reproducibility of 33 % or less. However, some levels of analyte above or below this value may have a relative standard deviation of reproducibility > 33 %; for details consult the respective annex. For quantitative methods, the LOD corresponds to the lowest level of analyte for which the relative standard deviation of reproducibility should be 33 % or less [2].

The practical LOD is the lowest relative quantity of the target DNA that can be detected, given a known (determined/estimated) number of target taxon genome copies. For the calculation of the copy numbers, based on the molecular mass of the respective species genome, the genome sizes as reported in

Reference [7] should be used. The practical LOD is related to the test portion, the quality/quantity of the template DNA, and the absolute LOD of the method.

For those matrices for which no validated method or associated LOD is available, laboratories shall report the practical LOD based on internal performance experience, or state it is unknown for these matrices and make reference to the validated LOD on a specific matrix.

4.3.3 Limit of quantitation (LOQ)

The LOQ values for each analytical method as specified in the annexes of ISO 21569, ISO 21570 and ISO 21572 are based on data from collaborative trial validation and/or claims from the method developer, and are included for information purposes only. The LOQ values reported from collaborative trial data generally refer to the lowest level of analyte that was observed to have a relative standard deviation of reproducibility of 25 % or less. However, some levels of analyte above or below this value may have a relative standard deviation of reproducibility > 25 %; for details consult the respective annex, and see References [2] and [6].

The practical LOQ is the lowest relative quantity of the target DNA that can reliably be quantified, given a known (determined/estimated) number of target taxon genome copies. For the calculation of the copy numbers, based on the molecular mass of the respective species genome, the genome sizes as reported in Reference [7] should be used. The practical LOQ is related to the test portion, the quality/quantity of the template DNA, and the absolute LOQ of the method.

For those matrices for which no validated method or associated LOQ is available, laboratories shall report the practical LOQ based on internal performance experience, or state it is unknown for these matrices and make reference to the validated LOQ on a specific matrix.

5 General laboratory and procedural requirements

5.1 General

The procedure includes the following steps:

— obtain a representative sample;

— homogenize the laboratory sample;

— reduce the laboratory sample to the test sample;

— prepare and grind the sample;

— extract the analyte;

— test, interpret and report the results.

Procedure-specific instructions are found within the main text and the individual annexes of ISO 21569, ISO 21570, ISO 21571 and ISO 21572.

5.2 Use of controls

The controls shall be used according to Table 1. This table is applicable to DNA-based methods only. Controls applicable to protein-based methods are described in ISO 21572.

Table 1 — Flow diagram showing intersection between successive steps and inclusion of controls[a]

Control step	Environment control [b]	Extraction blank control [c]	Positive extraction control [d]	Positive DNA target control [e]	Negative DNA target control [f]	Amplification reagent control [g]	PCR inhibition control [h]
Homogenization	recommended						
Nucleic acid extraction	↓[a]	one per series	mandatory at regular intervals				
Assessment of nucleic acid quality	↓	↓	↓				
Nucleic acid amplification	↓	↓	↓	mandatory	recommended	mandatory	recommended, but mandatory in certain cases[i]
Assessment of results of nucleic acid amplification	↓	↓	↓	↓	↓	↓	↓
Interpretation		↓	↓	↓	↓	↓	↓
Test report		↓	↓	↓	↓	↓	↓

[a] The arrows indicate that this control should be applied in the subsequent analytical steps.

[b] The use of environment controls will help the laboratory to identify sources of contamination at an early stage and can even be used to identify in which work area the contamination is present.

[c] At least one extraction blank control shall be included each time DNA is extracted from one or more samples. The tube shall always be the last in each series. It may be appropriate to put one extraction blank on e.g. a rack of eight tubes or a microplate of 96 wells for automated extraction.

[d] A positive extraction control should be included regularly, and always when a new batch of extraction reagents is used. This control will reveal if something is wrong with the reagents or the performance of the extraction protocol.

[e] The positive DNA target control demonstrates the ability of the nucleic acid amplification procedure to detect the nucleic acid representative of the GMO or target taxon. This condition can also be fulfilled by an appropriate positive extraction control.

[f] The negative DNA target control demonstrates the ability of the nucleic acid amplification procedure to avoid false positive amplification in the absence of the nucleic acid representative of the GMO or target taxon.

[g] The amplification reagent control demonstrates the absence of contaminating nucleic acid in the PCR reagent batches used. The amplification reagent control can be omitted when the extraction blank control is used.

[h] The PCR inhibition control may be used to demonstrate the absence of soluble inhibitors. This may also be demonstrated by serial dilutions of the template nucleic acid. However, some type of assessment of the effect of soluble inhibitors on the results of the analysis of the sample shall be made.

[i] A PCR-inhibition control is mandatory, if all PCR-tests on the sample are negative and for matrices where the yield of amplifiable DNA is not known.

5.3 Laboratory organization

5.3.1 General

Compliance with applicable requirements with respect to safety regulations and manufacturers' safety recommendations shall be followed and should be in accordance with the guidelines outlined in ISO/IEC 17025.

5.3.2 Laboratory design

Accidental DNA contamination can originate from dust and spreading aerosols. As a consequence, the organization of the work area in the laboratory is logically based on

— systematic containment of the methodological steps involved in the production of the results, and

— a “forward flow” principle for sample handling.

For DNA-based methods, the following laboratory design applies.

A minimum of four separately designated contained/dedicated work areas with their own apparatus are required, as follows:

a) a work area for grinding and homogenization;

b) a work area for extraction of the nucleic acid from the test material;

c) a work area dedicated to the set up of PCR/amplification reactions;

d) a work area dedicated to subsequent processing, including analysis and characterization of the amplified DNA sequences, if applicable.

If grinding techniques producing dust particles are used, this work shall be carried out in an additional work area.

Physical separation through the use of different rooms is the most effective and preferable way of ensuring separate work areas, but other methods may be used as a protection against contamination, provided their effectiveness is comparable.

5.3.3 Personnel

Staff shall wear different laboratory coats in different work areas, such as the grinding area or post-PCR area. They shall also wear disposable gloves. Where possible, the use of powdered gloves should be avoided for pre-PCR operations reactions, since the powder can inhibit PCR reactions or contaminate them when it is made from corn starch, for example. Gloves and laboratory coats should be changed at appropriate frequencies. All PCR procedures shall be carried out under non-contaminating conditions as far as is possible.

All personnel who perform steps in the testing procedure should be trained to work with the techniques as appropriate.

5.3.4 Apparatus and equipment

The laboratory should use properly maintained equipment suitable for the methods employed. In addition to standard laboratory equipment, additional apparatus is described in the annexes of the specific standards.

Apparatus and equipment shall be maintained according to manufacturers' instructions.

5.3.5 Materials and reagents

For the analysis, unless otherwise stated, use only analytical grade reagents suitable for molecular biology, free from DNA and DNases. Reagents and solutions should be stored at room temperature, unless otherwise specified. PCR reagents should be stored in small aliquots to minimize the risk of contamination. The water used shall be double-distilled or of comparable quality. Solutions should be prepared by dissolving the appropriate reagents in water and autoclaved, unless specified differently. Sterile filtration devices (possibly 0,22 µm pore size) may be used when autoclaving is not possible.

In order to avoid contamination, sterile technique should be adopted in the PCR set-up area; i.e. powder-free gloves, sterilized plastic ware, autoclaved reagents, disposable plastic ware, aerosol-protected pipette tips should be used.

Materials and all containers and disposables containing reagents shall be preserved from any contaminating agent (e.g. dust).

Manufacturers' recommendations for the use of reagents should be followed. Appropriate controls may be used to assess the integrity of reagents and the absence of DNase.

No unintended enzyme activities (e.g. exonuclease) that might interfere with PCR shall be present in the preparation. The reaction buffer shall be suitable for the brand of polymerase used.

6 Interpretation and expression of results

6.1 General

For quantitative methods, the calculation and expression of results is given in ISO 21570 and ISO 21572. No ambiguous results shall be expressed.

6.2 Interpretation of controls

Each control has a valid value and, if the observed result for any control is different from the valid value, the analysis shall be repeated. Environmental controls may be positive (amplification product of expected size detected) or negative (no amplification product detectable), but a positive result shall always initiate measures to remove and prevent contamination of the laboratory environment. If a non-valid result for any of the other controls is obtained repeatedly, measures shall be taken to locate and remove/replace the source(s) responsible for the error, and the analysis then repeated. Analytical results shall only be reported when all controls yield valid values. The valid values for the controls are as follows:

— positive extraction controls shall always be positive;

— extraction blank controls shall always be negative;

— positive DNA target controls shall always be positive;

— negative DNA target controls shall always be negative;

— amplification reagent controls shall always be negative.

PCR-inhibition controls shall not show significant inhibitory effects on the reaction (for qualitative analysis, the effect of inhibition may be less important than for quantitative analysis).

Possible PCR results of the controls are listed in Table 2. These are used for interpreting/reporting the test sample result.

Table 2 — Examples of PCR results

Test sample	Positive extraction control	Extraction blank control	Negative DNA target control	Positive DNA target control	Interpreted result
+[a]	+	–	–[b]	+	positive
–	+	–	–	+	negative
+	+	+	–	+	inconclusive [c]
–	–	+	–	–	inconclusive [c]
–	–	–	–	–	inconclusive [d]

a PCR product is detectable.

b No PCR product is detectable.

c The procedure is repeated beginning with the extraction step (possible contamination).

d The procedure is repeated using another extraction method or a further purification step (possible inhibition).

All testing steps and results shall be recorded.

6.3 Expression of a negative result

A negative result shall never be expressed as zero or “GMO not present”.

The following sentence shall appear in the test report:

> “For target analyte X, GMO-derived material was not detected.”

Furthermore, the test report shall include information on

— the LOD of the method validated on a specific matrix, and

— the practical LOD for the matrix based on laboratory experience or the sample analysed (or a statement that it is unknown).

The practical LOD should be greater than or equal to the LOD of the validated method.

6.4 Expression of a positive result

The following sentence or equivalent shall appear in the test report:

> “For target analyte X, the presence of material derived from (state specific target sequence) was detected.”

The identity of the GMO may be included, if known.

6.5 Expression of ambiguous results

Results from all test portions shall be consistent. When at least one test portion gives a positive result and at least one gives a negative result, the analysis shall be repeated.

If at least two repetitions of the procedure (beginning with the nucleic acid extraction) give ambiguous results such as a positive and a negative result, the report should state that the sample is negative at the limit of detection as expressed in ISO 21569 and ISO 21570.

6.6 Quality assurance requirements

General aspects of quality assurance are covered in ISO/IEC 17025.

When methods have been validated and demonstrated to be fit for purpose for a single matrix, these methods should not be applied to similar or other matrices prior to establishing the fitness for purpose within these additional matrices.

7 Test report

The test report shall contain at least the following information:

a) all information needed to identify the laboratory sample;

b) any particular information relating to the laboratory sample (e.g. insufficient size, degraded state);

c) reference to this International Standard and the respective annex(es) followed;

d) statement about date and type of sampling procedure(s) used;

e) date of receipt;

f) storage conditions, if applicable;

g) analysis start/end date, if applicable;

h) person responsible for the analysis;

i) size of the laboratory sample and test sample;

j) results according to the requirements of the specific method and the units used to report the results and the calibrators and the calculation method used;

k) any particular observations made during testing;

l) any deviations, additions to, or exclusions from, the test specification;

m) requirements as specified in the test report clause of ISO 21569 or ISO 21570;

n) any statements required as specified in Clause 6.

Information shall be given with regard to the units.

The measurement uncertainty and its level of confidence shall, on request, be made available to the user of the results.

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