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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

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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.