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

AMENDMENT 1

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

AMENDEMENT 1

Reference number
ISO 24276:2006/Amd.1:2013(E)

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Foreword

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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.

Amendment 1 to ISO 24276:2006 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

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

AMENDMENT 1

Page v, Introduction

Replace the existing text with the following.

> The purpose of an analysis for the detection of genetically modified organisms and derived products is to identify and optionally quantify genetic elements or proteins common to genetically modified organisms (GMOs) and their derived products in a given matrix.
>
> These steps are detailed in this International Standard and in the following documents:
>
> 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.

Page 1, Scope

Replace the existing first paragraph with the following.

> This International Standard specifies how to use the standards for 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.

Page 1, 3.1

Replace 3.1.2 to 3.1.26 with the following.

3.1.2
laboratory sample
sample received by the laboratory and intended for inspection or testing

NOTE Adapted from ISO 7002:1986,[9] A.19.

3.1.3
test sample
representative fraction of the laboratory sample to be ground

3.1.4
test portion
portion of the test sample as prepared for testing or analysis, the whole quantity being used for analyte extraction at one time

NOTE Adapted from ISO 6887-2:2003,[5] 3.2.

3.1.5
specificity
property of a method to respond exclusively to the characteristic or analyte under investigation

3.1.6
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.

3.1.7
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.

3.1.8
limit of quantification
LOQ
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.

3.1.9
accuracy
closeness of agreement between a test result and the accepted reference value

[ISO 5725-1:1994, 3.6].

3.1.10
trueness
closeness of agreement between the average value obtained from a large series of test results and an accepted reference value

NOTE 1 The measure of trueness is usually expressed in terms of bias. Trueness has been referred to as "accuracy of the mean".

NOTE 2 Adapted from ISO 5725-1:1994, 3.7.

3.1.11
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.

[ISO 5725-1:1994, 3.12]

3.1.12
repeatability
precision under repeatability conditions

[ISO 5725-1:1994, 3.13]

3.1.13
reproducibility
precision under reproducibility conditions

[ISO 5725-1:1994, 3.17]

3.1.14
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

[ISO 5725-1:1994, 3.14]

3.1.15
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

[ISO 5725-1:1994, 3.18]

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 should be explicitly stated.

3.1.16
repeatability standard deviation
standard deviation of test results obtained under repeatability conditions

[ISO 5725-1:1994, 3.15]

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.

3.1.17
reproducibility standard deviation
standard deviation of test results obtained under reproducibility conditions

[ISO 5725-1:1994, 3.19]

NOTE Reproducibility standard deviation is a measure of the dispersion of the distribution of test results under reproducibility 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.

3.1.18
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*.

[ISO 5725-1:1994, 3.16]

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

3.1.19
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*.

[ISO 5725-1:1994, 3.20]

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

3.1.20
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[8] and the ISO/AOAC/IUPAC harmonized protocol (Reference [6]).

3.1.21
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 (Reference [3]).

3.1.22
practicability
ease of operations, in terms of sample throughput and costs, to achieve the required performance criteria and thereby meet the specified purpose

3.1.23
applicability range
range of quantification/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.

3.1.24
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

3.1.25
screening method
method that rapidly and reliably eliminates (screens) a large number of negative (or positive) test samples and restricts 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 detects gene products (such as proteins) or genetic elements (such as promoters, terminators, or other genetic elements of interest) common to several GMOs.

3.1.26
construct-specific method
method that targets a combination of inserted DNA sequences that are only found in GMO-derived material

3.1.27
event-specific method
method that detects a specific sequence that is only present in a specific transformation event

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

Page 8, Figure 1

Replace Figure 1 with the following.

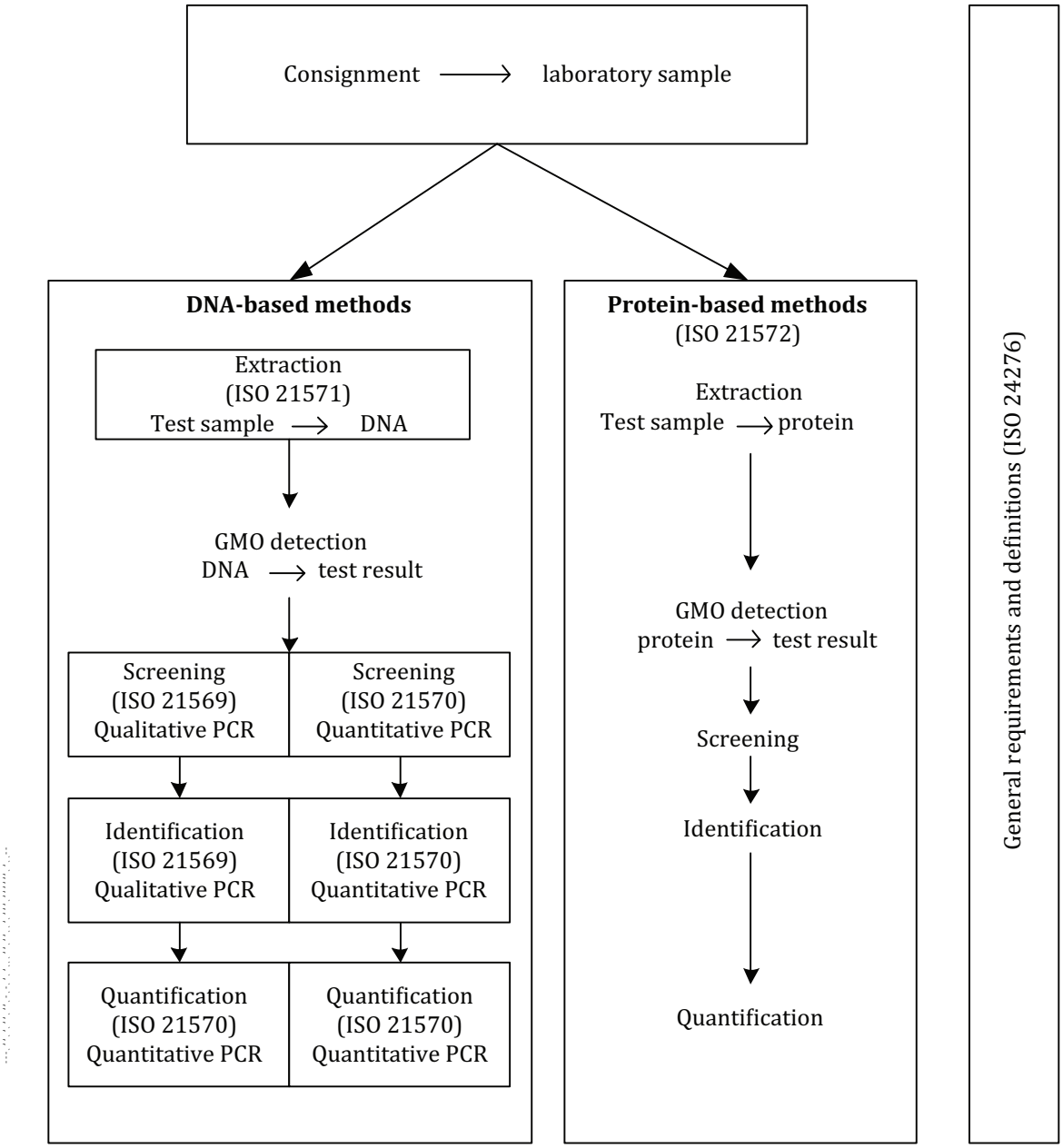

Figure 1 — Flow diagram of interrelationships of International Standards on GMO detection methods

Page 9, 4.3

Replace 4.3.2 and 4.3.3 with the following.

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 less than or equal to 5 %.

4.3.3 Limit of quantification (LOQ)

The LOQ values for each analytical method as specified in the annexes of 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.

Page 10, 5.1 and 5.2

Replace the existing text with the following.

5.1 General

The procedure includes the following steps:

— preparation of the test sample (*optional* with the customer agreement: if the test sample is not the whole laboratory sample, homogenize the laboratory sample and obtain test samples in accordance with the relevant International Standards);

— grinding of the test sample;

— preparation of test portions;

— extraction of the analyte;

— testing, interpretation, and reporting of 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. Table 1 is applicable to DNA-based methods only. Controls applicable to protein-based methods are described in ISO 21572.

Table 1 — Flow diagram showing intersection of successive steps and inclusion of controls

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	Mandatory	—	—	—	—	—	—
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 helps 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. This can be demonstrated in various ways, e.g. if negative samples included in the series of homogenized samples showed negative results, starting at the first step of the process (e.g. grinding step if relevant)

[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, for example, 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 reveals 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.

Page 12, 5.3.4

Replace the existing text with the following.

> 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 guidelines outlined in ISO/IEC 17025;[10] manufacturers´ instructions should be taken into consideration.

Page 14, Table 2

Replace the existing Table 2 with the following.

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 within the detection limit of analytical method used and test portion analysed.

[b] No PCR product is detectable within the detection limit of analytical method used and test portion analysed.

[c] The procedure shall be repeated beginning with the extraction step (possible contamination).

[d] The procedure shall be repeated using another extraction method or a further purification step (possible inhibition).

Page 14, 6.3 to 6.5

Replace the existing text with the following.

6.3 Expression of a negative result

The following text shall appear in the test report:

> "For sample X, target sequence Y was not detected.
>
> The LOD of the method is *x* % determined with ABC (identify the reference material)."

If it cannot be demonstrated that the amount of target DNA included in the PCR is sufficient for the LOD to be applicable, then the following sentence shall be added:

> "However, the amount of the target DNA extracted from species X may be/was insufficient for the LOD to be applicable for this sample."

In addition, if applicable: "The practical limit of detection is *x* %".

NOTE The practical LOD of the sample is determined by the quantity of DNA of the species included in the analytical reaction (copy number), and the ratio relative to the absolute LOD of the GM target (copy number).

6.4 Expression of a positive result

In case of a qualitative analysis, the following text shall appear in the test report:

> "For sample X, target sequence Y was detected."

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

In case of a quantitative analysis:

— if the target taxon-specific sequence and the GM target sequence are both detected but the quantity is below the LOQ of at least one of the target sequences, the following text shall appear in the test report for each GMO sequence:

> "GMO (specify the GMO) derived DNA as determined by detection of (specify target sequence) derived from (specify species) was detected, below the practical limit of quantification"

In addition, if applicable: "The practical limit of quantification is x %."

— if the target taxon-specific sequence and the GM target sequence are both detected and the quantity is above the LOQ for both target sequences, for each GMO, state:

"The content of GMO (specify the GMO) derived DNA as determined by detection of (specify target sequence) derived from (specify species) is $x \pm u_{meas}$ %"

where u_{meas} is the measurement uncertainty.

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 one repetition of the procedure, beginning with the nucleic acid extraction, gives ambiguous results such as a positive and a negative result, the report should state that the sample is negative at the limit of detection.

Results within the same test portion shall be consistent. In case of +/− results for the two replicates, repeat the two PCR for the respective test portion. If the two novel replicates are tested +/− or −/−, the test portion is considered as negative.

Results are expressed as in ISO 21569 and ISO 21570.

Page 15, Test report

Replace the existing text with the following.

The test report shall be signed by an authorized person in accordance with ISO/IEC 17025[10] and shall contain at least the following information:

a) all information needed to identify the laboratory sample (including size of the laboratory sample);

b) any particular information relating to the laboratory sample;

c) All information related to the test sample (size of the sample ground);

d) reference to this International Standard (ISO 24276:2006) and the relevant annex(es) followed;

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

f) date of receipt;

g) storage conditions, if applicable;

h) analysis start and end dates, if applicable;

i) person responsible for the analysis;

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;

The test report shall be signed by an authorized person in accordance with ISO/IEC 17025[10] and shall contain at least the following information:

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.

Page 16, Bibliography

Replace Reference [1] with the following.

[1] Codex Alimentarius Commission. General criteria for the selection of methods of analysis using the criteria approach.In: *Procedural manual*, 19th edition, p. 51. Rome: FAO, WHO, 2010

Replace References [5] and [6] with the following.

[5] ISO 6887-2:2003, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

[6] Thompson, M., Ellison, S.L., Wood, R. Harmonized guidelines for single laboratory validation of methods of analysis. *Pure Appl. Chem.* 2002, **74**, pp. 835–855

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