INTERNATIONAL STANDARD

ISO 21572

Second edition
2013-02-01

Foodstuffs — Molecular biomarker analysis — Protein-based methods

Produits alimentaires — Analyse des biomarqueurs moléculaires — Méthodes basées sur les protéines

Reference number
ISO 21572:2013(E)

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

Published in Switzerland

Contents

Page

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 21572 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

This second edition cancels and replaces the first edition (ISO 21572:2004), which has been technically revised. It also incorporates the Technical Corrigendum ISO 21572:2004/Cor.1:2005.

Foodstuffs — Molecular biomarker analysis — Protein-based methods

WARNING — Follow all instructions provided by the kit/reagent manufacturers and other standard laboratory safety procedures. Read and implement the material safety data sheets (MSDS).

1 Scope

This International Standard provides general guidelines and performance criteria for methods for the detection and/or quantification of specific proteins or protein(s) of interest [POI(s)] in a specified matrix.

These general guidelines address existing antibody based methods. Methods other than those described in Annex A or Annex B can also detect the POI. The same criteria as outlined in this International Standard apply generally.

2 Normative references

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

ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 24276 and the following apply.

3.1 General

3.1.1
sample
subset of a population made up of one or more sampling units

[SOURCE: ISO 3534-2:2006, 1.2.17]

3.1.2
laboratory sample
sample (3.1.1) as prepared for sending to the laboratory and intended for inspection or testing

[SOURCE: ISO 78-2:1999, 3.1]

3.1.3
test sample
sample (3.1.1) as prepared for testing or analysis, the whole quantity or part of it being used for testing or analysis at one time

[SOURCE: ISO 3534-2:2006, 5.3.11]

3.1.4
test portion
part of a *test sample* (3.1.3) which is used for testing or analysis at one time

[SOURCE: ISO 3534-2:2006, 5.3.12]

3.1.5
matrix
products submitted for analysis, which might have differences in chemical composition and physical state

[SOURCE: ISO 22174:2005, 3.1.4]

3.1.6
denaturation of proteins
physical and/or (bio)chemcial treatment which destroys or modifies the structural, functional, enzymatic, or antigenic properties of the POI or the analyte

3.2 Terms relating to antibodies

3.2.1
antibody
protein (immunoglobulin) produced and secreted by B lymphocytes in response to a molecule recognized as foreign (antigen) and capable of binding to that specific *antigen* (3.2.2)

3.2.2
antigen
substance that stimulates the production of *antibodies* (3.2.1) and reacts with them

3.2.3
clone
population of identical cells derived from a single cell

3.2.4
cross-reactivity
binding of the *antibody* (3.2.1) to substances other than the analyte of primary interest

3.2.5
monoclonal antibody
antibody (3.2.1) produced from a single hybridoma *clone* (3.2.3) and directed to a single *antigen* (3.2.2) determinant

3.2.6
polyclonal antibody
mixture of immunoglobulin molecules, secreted against a specific immunogenic substance, each recognizing a different epitope

[SOURCE: ISO 19001:2013, 3.11]

3.2.7
selectivity of an antibody
ability of an *antibody* (3.2.1) to specifically bind to an *antigen* (3.2.2) determinant and not to other similar structures or other antigens

3.3 Terms relating to techniques

3.3.1
conjugate
material produced by attaching two or more substances together by covalent bond via chemical groups

EXAMPLE Conjugates of antibodies with fluorochromes (e.g. chemical entity, such as a molecule or group, which emits light that is in response to being stimulated by absorption of incident light), radiolabelled substances, gold or enzymes are often used in immunoassays.

3.3.2
western blotting protocol
protein immunoblot
transfer of a protein to a binding surface following separation by electrophoresis that may be visualised using a variety of methods

EXAMPLE One example of such a method is with a specific radiolabelled or enzyme-conjugated antibody followed by the addition of an enzyme-specific substrate to form a coloured reaction product.

3.3.3
enzyme linked immunosorbent assay
ELISA
in vitro assay used for qualitative, semi-quantitative, or quantitative purposes that combines enzyme-linked antibodies and a substrate to form a coloured reaction product

3.3.4
test kit
set of chemicals, materials and instructions for use, packaged together and intended for use as specified by the manufacturer of the kit

3.3.5
lateral flow immunochromatographic assay
lateral flow device/strip
qualitative or semiquantitative, simple rapid assay formats intended to detect the presence (or absence) of a POI in a sample where an *antibody* (3.2.1) or an analyte is coated to a solid surface and dipped into a test liquid to provide a measure of the POI in the liquid

Note 1 to entry: The test sample flows along a solid substrate via capillary action. After the liquid sample enters the test strip, it encounters a coloured reagent which mixes with the sample and transits the substrate encountering lines or zones which have been pretreated with an antibody or antigen. Depending on the analytes present in the sample the coloured reagent can become bound at the test line or zone. These assays can operate as either competitive or sandwich assays.

3.4 Terms relating to control

3.4.1
reference material
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process or in examination of nominal properties

[SOURCE: ISO/IEC Guide 99]

3.4.2
measurement standard
measured material, measuring instrument, *reference material* (3.4.1) or measuring system intended to define, realize, conserve or reproduce one or more values to serve as a reference or preparation of known characteristics used to standardize the analysis

3.5 Terms relating to validation

3.5.1
accuracy
closeness of agreement between a test result or measurement result and a reference value

Note 1 to entry: The term “accuracy”, when applied to a set of test results or measurement results, involves a combination of random components and a common systematic error or *bias* (3.5.3) component.

Note 2 to entry: When applied to a test method, the term accuracy refers to a combination of trueness and *precision* (3.5.2).

[SOURCE: ISO 3534-2:2006, 3.3.1, modified — Notes 1 and 2 differ from the original and there is no Note 3.]

3.5.2
precision
closeness of agreement between independent test/measurement results obtained under stipulated conditions

Note 1 to entry: Precision is normally expressed in terms of standard deviation.

[SOURCE: ISO 3534-2:2006, 3.3.4]

3.5.3
bias
difference between the expectation of a test result or measurement result and a true value

Note 1 to entry: Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias. A larger systematic difference from the true value is reflected by a larger bias value.

[SOURCE: ISO 3534-2:2006, 3.3.2]

3.5.4
sensitivity
quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured

Note 1 to entry: The sensitivity can depend on the value of the quantity being measured. Sensitivity usually is meant as the smallest quantity or concentration of the analyte that can be reliably distinguished from background.

[SOURCE: ISO/IEC Guide 99]

3.5.5
selectivity
extent to which a method can determine particular analyte(s) in a mixture(s) or matrice(s) without interferences from other components of similar behaviour

Note 1 to entry: Selectivity is the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence of other components. Selectivity can be graded.

[SOURCE: Pure Appl. Chem.]

3.5.6
detection limit
limit of detection
LOD
lowest concentration or content of the POI per defined amount of matrix that can be consistently detected under the experimental conditions specified in the method

[SOURCE: ISO 22174:2005, 3.1.8, modified — “LOD” has been added and “of the target organism” became “of the POI”.]

3.5.7
determination limit
limit of quantification
LOQ
lowest concentration or content of the POI per defined amount of matrix that can be measured with reasonable statistical certainty consistently under the experimental conditions specified in the method

3.5.8
applicability range
range of quantification
range of linearity
dynamic range
upper and lower limits of quantification as expressed by a set of reference materials (or dilutions) with a suitable level of precision and *accuracy* (3.5.1)

3.5.9
repeatability conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time

Note 1 to entry: Repeatability conditions include: the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time.

[SOURCE: ISO 3534-2:2006, 3.3.6, modified — the Note has been deleted.]

3.5.10
repeatability
precision under *repeatability conditions* (3.5.9)

[SOURCE: ISO 3534-2:2006, 3.3.5]

3.5.11
repeatability limit
r
value less than or equal to which the absolute difference between two test results obtained under *repeatability conditions* (3.5.9) may be expected to be with a probability of 95 %

[SOURCE: ISO 5725-1:1994, 3.1.6]

3.5.12
repeatability standard deviation
standard deviation of test results or measurement results obtained under *repeatability conditions* (3.5.9)

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under repeatability conditions.

Note 2 to entry: Similarly, "repeatability variance" and "repeatability coefficient of variation" can be defined and used as measures of the dispersion of test or measurement results under repeatability conditions.

[SOURCE: ISO 3534-2:2006, 3.3.7]

3.5.13
reproducibility conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment

[SOURCE: ISO 3534-2:2006, 3.3.11]

3.5.14
reproducibility
precision under *reproducibility conditions* (3.5.13)

[SOURCE: ISO 3534-2:2006, 3.3.10; ISO 78-2:1999, 3.13]

3.5.15
reproducibility limit
R
value less than or equal to which the absolute difference between two test results obtained under *reproducibility conditions* (3.5.13) may be expected to be with a probability of 95 %

[SOURCE: ISO 5725-1:1994, 3.20]

3.5.16
reproducibility standard deviation
standard deviation of test results or measurement results obtained under *reproducibility conditions* (3.5.13)

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under reproducibility conditions.

Note 2 to entry: Similarly, "reproducibility variance" and "reproducibility coefficient of variation" can be defined and used as measures of the dispersion of test or measurement results under reproducibility conditions.

[SOURCE: ISO 3534-2:2006, 3.3.12]

4 Principle

The POI is extracted according to the procedure described for that specific matrix, and a specific antibody is used to detect or measure the concentration of the POI in the sample. For the detection of specific proteins in ingredients, the basic principle of a protein-based method is to:

— take a representative sample of the matrix;

— extract the proteins;

— detect and/or quantify the POI derived from the matrix under study.

5 Reagents

During the analysis, use only reagents of recognized analytical grade and only deionized or distilled water or water that has been purified, or equivalent, unless indicated otherwise by the manufacturer of the reagents or the kit.

Other reagents, such as antibodies, conjugates, substrates, stop solutions and buffer components are method specific. Please refer to the method for specifics regarding reagents, such as protein standards or reference materials, antibodies or pre-coated solid surfaces, controls and samples.

Reagents are specified in A.4.2, A.4.3, B.4.2 and B.4.3.

6 Laboratory equipment

Laboratory equipment is specified in A.5 and B.5.

7 Sampling

Sampling is not part of the method specified in this International Standard, although Annex A and Annex B do provide sampling instructions according to the relevant methods. It is recommended that the parties concerned come to an agreement on this subject.

8 Procedure

8.1 General

Storage conditions and shelf-life of lateral flow strips, antibodies, conjugates, substrates, etc. shall be clearly specified by the provider.

Use appropriate laboratory equipment with low protein binding capacity (e.g. polypropylene tubes) to prevent protein adsorption during the whole procedure.

For the use of this International Standard, general requirements of quality assurance for laboratories shall be observed (e.g. concerning calibration of apparatus, double determination, blanks, use of reference materials, preparation of calibration curves). Carefully clean all equipment coming into direct contact with the sample to prevent contamination. See ISO/IEC 17025 for more information.

8.2 Preparation of sample solution

Once a representative sample is obtained, specific sample preparation procedures may be found in the annexes.

Grind samples as specified in the method before test portions are taken, if necessary. Powders/flour might have swelling properties and may require more extraction solution if a manufacturer's method does not specify this information. If the sample is not immediately used, follow your laboratory's procedure for storage (e.g. –20 °C or below).

Laboratory samples containing high amounts of fat may be nonhomogeneous and a larger test sample should be extracted. If applicable, instructions may be found in the annexes.

Weigh an appropriate amount (as specified in the relevant annex) of a representative test sample for analysis to create a test portion for extraction. Add extraction solution and homogenize or mix.

8.3 Extraction

Use an extraction procedure suitable for the matrix. Details of appropriate conditions for the extraction/dilution of the test portions, controls and reference materials are provided in Annex A for ELISA and Annex B for lateral flow strips. Care should be taken to use extraction procedures validated for the matrix. Extracted samples should be immediately used or treated as specified in the procedure for storage.

8.4 Preparation of calibration curves, positive controls and reference materials

For the preparation of calibration curves, positive controls and reference materials for Annex A, it is recommended to use matrix matched reference materials or reference materials that have been validated for the matrix. Calibration curves are not routinely required for qualitative application such as lateral flow strips, however, positive and negative controls can be prepared at the discretion of the analyst.

8.5 Assay procedure

For a quantitative test, select the required number of wells, (e.g. in ELISA) for the test portion(s) to be analysed, including blanks, measurement standards, and add each of them at minimum in duplicate, properly diluted to the assay.

For a qualitative test or semiquantitative test, select the required number of test (e.g. lateral flow strips or ELISA) needed for the test portions to be analysed. The stability of the final signal can vary. Read the results in a timely manner as specified in the annexes.

According to the method chosen, follow the instructions of each method for sample analyses, including blanks and measurement standards (if necessary). Allow the reaction to occur at a specified temperature range and time. If necessary, terminate the reaction according to the method described in the relevant

annex. For example, if ELISA method requires acquiring data on a spectrophotometer, perform this step. In the case of qualitative tests, generally these are interpreted visually, follow the kit instructions.

9 Interpretation and expression of results

9.1 General

The parameters to interpret vary depending on whether the assay is qualitative, semiquantitative or quantitative.

For quantitative methods, the coefficient of variation (CV) of optical density values resulting from replicate measurements of a sample test solution, in general, should not exceed 15 %. The CV of calculated concentrations resulting from replicate measurements of a sample test solution, in general, should not exceed 20 %.

If the CV limit is exceeded, the analyses should be repeated on freshly prepared sample test solution. To establish a CV, in this case, at least three determinations shall be carried out (e.g. values from three microtitre wells).

Negative results shall be reported as "negative at the limit of detection" and the limit of detection shall be reported.

Positive results below the limit of quantification shall be reported as "positive above the limit of detection, but below the limit of quantification". The limits of quantification and detection shall be reported.

9.2 Quantitative and semiquantitative analysis

The following parameters shall be evaluated: raw data of sample test solution, blanks, reference materials or measurement standards, and negative controls; percentage CV between replicates, percentage CV of standard and percentage CV of control samples.

According to ISO/IEC 17025:2005, 5.10.3.1 c), measurement uncertainty should be reported where applicable.

Quantitative results shall not be reported by extrapolating above the highest or below the lowest calibration point.

9.3 Qualitative analysis

For qualitative tests, including all applications thereof, the corresponding parameters are described in the annexes. The limit of detection shall always be reported and negative results shall be reported as "negative at the limit of detection".

Positive results shall also report the limit of detection.

10 Specific parameters which may influence results

10.1 General

The performance criteria listed in the method of Annex A are a set of performance specifications established for each method during the development, validation and routine use of the method. These parameters shall be estimated and evaluated for each method and are reliable and of consistently high quality. Each time a method is implemented the data generated shall be evaluated and compared with the established method performance criteria.

When a value (e.g. CV of replicate determinations) does not agree with the assay specifications, it signals that the result is atypical and warrants closer evaluation of the data. The list of specifications shall be taken as whole, individual parameters may in certain instances not meet the specifications, but the data may still be perfectly acceptable. If any of the criteria are not met, it should, however, be acknowledged in writing and the data evaluated to determine if the analysis of results should be adjusted, or if a particular

