

BS EN ISO 21572:2013



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Foodstuffs — Molecular biomarker analysis — Protein-based methods

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Foreword

This document (EN ISO 21572:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2013, and conflicting national standards shall be withdrawn at the latest by August 2013.

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The text of ISO 21572:2013 has been approved by CEN as EN ISO 21572:2013 without any modification.

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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)chemical 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.