BRITISH STANDARD

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Foodstuffs — Detection of food allergens — General considerations and validation of methods

ICS 67.050

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British Standards

National foreword

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Foreword

This document (EN 15842:2010) has been prepared by 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of an identical text or by endorsement, at the latest by August 2010, and conflicting national standards shall be withdrawn at the latest by August 2010.

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Introduction

The main focus of this European Standard is on immunoassays, chromotographic and nucleic acid based methods for the determination of food allergens. However, because of the rapid developments in this area, other technologies may be considered.

The search for food allergens is performed by means of the following successive (or simultaneous) steps. After sample collection, proteins, nucleic acids or other markers are extracted from the test portion. Extracted analytes can be further purified, simultaneously or after the extraction process. Afterwards, they are diluted (if necessary) and subjected to analytical procedures such as immunoassays (e.g. ELISA), nucleic acid based assays (e.g. PCR) or chromatographic (e.g. LC-MS).

These steps are detailed in this document and in the following documents:

EN 15633-1:2009, *Foodstuffs — Detection of food allergens by immunological methods — Part 1: General considerations*

EN 15634-1:2009, *Foodstuffs — Detection of food allergens by molecular biological methods — Part 1: General considerations*

1 Scope

This European Standard specifies how to use the standards for immunoassays, nucleic based and chromatographic methods and their relationship in the analysis of food allergens; and contains general definitions, requirements and guidelines for laboratory set-up, method validation requirements, description of methods, and test reports.

This document also specifies general guidelines for the requirements and use of reference materials for the determination of allergenic commodities in food products. The term "reference materials" in this document includes certified reference materials as well as quality control materials. Currently only a limited number of reference materials for food allergen determination are available. As new materials become accepted and validated, they may be appended as an annex to this document.

This document does not deal with sampling issues. It simply details processes involved from receipt of the laboratory sample to the end result.

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.

EN ISO/IEC 17025, *General requirement for the competence of testing and calibration laboratories (ISO/IEC 17025:2005)*

EN ISO 17511:2003, *In vitro diagnostic medical devices — Measurement of quantities in biological samples — Metrological traceability of values assigned to calibrators and control materials (ISO 17511:2003)*

ISO Guide 31, *Reference materials — Contents of certificates and labels*

ISO Guide 35, *Reference materials — General and statistical principles for certification*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
accepted reference value
value that serves as an agreed-upon reference for comparison and which is derived as:

— theoretical or established value, based on scientific principles,

— an assigned value, based on experimental work of some national or international organization,

— consensus value, based on collaborative experimental work under the auspices of a scientific or engineering group

[ISO Guide 30:1992]

3.2
accuracy
closeness of agreement between a test result or measurement result and the true value

NOTE 1 In practice, the accepted reference value is substituted for the true value.

NOTE 2 The term "accuracy", when applied to a set of test or measurement results, involves a combination of random components and a common systematic error or a bias component.

NOTE 3 Accuracy refers to a combination of trueness and precision.

[ISO 3534-2:2006]

3.3
applicability range
quantity interval within which the analytical procedure has been demonstrated by collaborative trial or other appropriate validation to have a suitable level of precision and accuracy

[EN ISO 24276:2006]

3.4
bias
difference between the expectation of a test result or measurement result and a true value

NOTE 1 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 accepted reference value is reflected by a larger bias value.

NOTE 2 The bias of a measuring instrument is normally estimated by averaging the error of indication over an appropriate number of repeated measurements. The error of indication is the "indication of a measuring instrument minus a true value of the corresponding input quantity".

[ISO 3534-2:2006]

3.5
Certified Reference Material
CRM
reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence

[ISO Guide 30:1992]

3.6
certified value
for a CRM, value that appears in the certificate accompanying the material

[ISO Guide 30:1992]

3.7
characterization
for a reference material, determination of one or more physical, chemical, biological, or technological property values that are relevant to its intended end use

[ISO Guide 30:1992]

3.8
collaborative study
interlaboratory study
interlaboratory study in which each laboratory uses a defined method of analysis to analyse identical portions of homogenous material to assess the performance characteristics obtained for the method of analysis

NOTE Guidelines for performing collaborative trials are elaborated in ISO 5725-1 [3] and in IUPAC harmonized protocol 1995 [16].

3.9
commutability of a material
closeness of agreement between the mathematical relationship of the measurement results obtained by two measurement procedures for a stated quantity in a given material, and the mathematical relationship obtained for the quantity in routine samples

[EN ISO 17511:2003]

3.10
consensus value (of a given quantity)
for a reference material, value of the quantity obtained by interlaboratory testing, or by agreement between appropriate bodies or experts

[ISO Guide 30:1992]

3.11
fitness for purpose
applicability
degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose

NOTE See [17].

3.12
homogeneity
condition of being of uniform structure or composition with respect to one or more specified properties

NOTE A reference material is said to be homogeneous with respect to a specified property if the property value, as determined by tests on samples of specified size, is found to lie within the specified uncertainty limits, the samples being taken either from different supply units (bottles, packages, etc.) or from a single supply unit.

[Adapted from ISO Guide 30:1992]

3.13
laboratory sample
sample as prepared for sending to the laboratory and intended for inspection or testing

[ISO 78-2:1999]

3.14
limit of detection
LOD
minimum amount or concentration of the analyte in test sample which can be detected reliably but not necessarily quantified, as demonstrated by a collaborative trial or other appropriate validation

3.15
limit of detection for quantitative determinations
amount of an analyte corresponding to the lowest measurement signal which with a closely defined confidence may be interpreted as indicating that the analyte is present in the sample, but without allowing exact quantification

3.16
limit of detection for qualitative determinations
threshold concentration below which positive identification is unreliable according to the established requirements for reliability

NOTE See [24].

3.17
limit of quantitation
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 collaborative trail or other appropriate validation

NOTE See [24].

3.18
limit of quantification
limit of determination
lowest amount of an analyte which can be determined quantitatively with a closely defined confidence

NOTE See [24].

3.19
linearity
ability to elicit test results that are directly, or by means of well defined, mathematical transformations, proportional to the concentration of analyte in samples within a given range

NOTE See [15].

3.20
matrix
all compounds in the sample with the analyte

NOTE Each matrix has generally a common name which permits classification.

[EN ISO 21572:2004]

3.21
outlier
member of a set of values which is inconsistent with the other members of that set

NOTE ISO 5725 specifies the statistical tests and the significance level used to identify outliers in trueness and precision experiments.

[ISO 5725-1:1994]

3.22
practicability
ease of operations, in terms of sample throughput and costs, to achieve the required performance criteria and thereby meet the specified purpose

[EN ISO 24276:2006]

3.23
precision
closeness of agreement between independent test/measurement 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 the specified value.

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

NOTE 3 Quantitative measures of precision depend critically on the stipulated conditions. Repeatability conditions and reproducibility conditions are particular sets of extreme stipulated conditions.

[ISO 3534-2:2006]

3.24
primary standard
standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to other standards of the same quantity, within a specified context

[ISO Guide 30:1992]

3.25
recovery
proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is extracted and presented for measurement

NOTE See [18].

3.26
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 30:1992]

3.27
reference method
thoroughly investigated method, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have accuracy and precision commensurate with its intended use and that can therefore be used to assess the accuracy of other methods for the same measurement, particularly in permitting the characterisation of a reference material

[ISO Guide 30:1992]

3.28
repeatability
precision under repeatability conditions

NOTE Repeatability can be expressed quantitatively in terms of the dispersion characteristics of the results.

[ISO 3534-2:2006]

3.29
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 Repeatability conditions include:

— same measurement procedure or test procedure;

— same operator;

— same measuring or test equipment used under the same conditions;

— same location;

— repetition over a short period of time.

[ISO 3534-2:2006]

3.30
repeatability limit
r
repeatability critical difference for a specified probability of 95 %

[ISO 3534-2:2006]

3.31
repeatability standard deviation
standard deviation of test results or measurement results obtained under repeatability conditions

NOTE 1 It is a measure of the dispersion of the distribution of test or measurement results under repeatability conditions.

NOTE 2 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.

[ISO 3534-2:2006]

3.32
reproducibility
precision under reproducibility conditions

NOTE 1 Reproducibility can be expressed qualitatively in terms of the dispersion characteristics of the results.

NOTE 2 Results are usually understood to be corrected results.

[ISO 3534-2:2006]

3.33
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 by different operators using different equipment

[ISO 3534-2:2006]

3.34
reproducibility limit
reproducibility critical difference for a specified probability of 95 %

[ISO 3534-2:2006]

3.35
reproducibility standard deviation
standard deviation of test results or measurement results obtained under reproducibility conditions

NOTE 1 It is a measure of the dispersion of the distribution of test or measurement results under reproducibility conditions.

NOTE 2 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.

[ISO 3534-2:2006]

3.36
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 See [25].

3.37
secondary standard
standard whose value is assigned by comparison with a primary standard of the same quantity

NOTE 1 Most CRMs fall into this category since the certification of property values is usually carried out by a procedure traceable to primary standards. The position of a CRM in the measurement hierarchy is no indication of its suitability for a particular purpose. Thus for the determination of an analyte in a food matrix, secondary standards, which contain e.g. the allergenic food in a similar state of chemical combination and in a similar matrix to the test sample, should be greatly preferred to primary standards of pure materials.

NOTE 2 See [14].

3.38
selectivity
extent to which a method can determine particular analyte(s) in mixtures or matrices without interferences from other components

NOTE See [15].

3.39
sensitivity
change in the response divided by the corresponding change in the concentration of a standard (calibration) curve

NOTE See [15].

3.40
specificity
property of a method to respond exclusively to the characteristic or analyte under investigation

NOTE See [15].

3.41
stability
ability of a reference material, when stored under specified conditions, to maintain a stated property value within specified limits for a specified period of time

[ISO Guide 30:1992]

3.42
traceability
property of the result of a measurement or the value of a standard whereby it can be related, with a stated uncertainty, to stated references, usually national or international standards, through an unbroken chain of comparisons

NOTE 1 The concept is often expressed by the adjective "traceable".

NOTE 2 The unbroken chain of comparisons is called a traceability chain.

[ISO Guide 30:1992]

3.43
test kit
set of components and instructions for use, packed together and intended for in vitro measurement or detection of a specified analyte including sample preparation

3.44
test portion
quantity of material drawn from the test sample (or from the laboratory sample if both are the same) and on which the test or observation is actually carried out

[ISO 78-2:1999]

3.45
test sample
sample prepared from the laboratory sample and from which test portions will be taken

[ISO 78-2:1999]

3.46
trueness
closeness of agreement between the expectation of a test result or a measurement result and a true value

NOTE 1 The measurement of trueness is usually expressed in terms of bias.

NOTE 2 Trueness is sometimes referred to as "accuracy of the mean". This usage is not recommended.

NOTE 3 In practice, the accepted reference value is substituted for the true value.

[ISO 3534-2:2006]

3.47
uncertified value
value of a quantity, included in the certificate of a CRM or otherwise supplied, which is provided for information only but is not certified by the producer or the certifying body

[ISO Guide 30:1992]

3.48
uncertainty of a certified value
estimate attached to a certified value of a quantity which characterizes the range of values within which the "true value" is asserted to lie with a stated level of confidence

[ISO Guide 30:1992]

4 General aspects for the use of reference materials in food allergen analysis

4.1 Reference material

The reference materials described hereafter are primarily intended for the calibration and validation of analytical methods for the determination of allergenic commodities in food products and not necessarily for the determination of a specific allergen. A metrological traceability chain as described in EN ISO 17511 shall be established for assigning certified values to matrix samples. This traceability chain shall include well characterised primary standards, which are designated or widely acknowledged as having the highest metrological quality. Standards of lower metrological order (secondary standards, matrix materials) should be traceable to the primary standard, but need to be commutable to the samples to be analysed (concerning expected concentration of the analyte, matrix and processing history, etc.). Matrix reference materials (secondary standards), which contain the analyte (allergenic food) in a similar chemical state and in a matrix

similar to the test sample, are needed for the calibration and validation of routine testing methods. Procedures for developing in-house reference materials have been proposed by IUPAC [17].

Generally, the homogeneity and stability of any reference material should be guaranteed and stated.

It is recommended to provide as much information about the origin and features of the reference material as possible (e.g. processing history, variety, geographical origin, preparation protocol, etc.).

4.2 Reference method

A reference method for the determination of allergenic food commodities should be independent of any technological influences and matrix effects, in order to reliably measure one or more property values characteristic of the allergen concerned. The reference method's accuracy and precision should be commensurate with its intended use and permit the characterization of a reference material.

Reference materials may be used to calibrate instruments, to determine trueness/bias of a method (validate a method) and to assign values to testing materials. In case of bias, checking the results obtained from a CRM by using a certain analytical method may be compared to the certified property value. If the absolute difference of the two sets of values (obtained values and certified value) exceeds the expanded uncertainty of this difference, the analytical method is considered to be biased (one or more systematic errors occurred). See also ISO Guide 32 [11] and ISO Guide 33 [12].

4.3 General requirements for production and storage of reference materials

The reference material producer shall identify and plan those processes which directly affect the quality of reference material production and shall ensure that they are carried out in accordance with specified procedures (e.g. ISO Guide 35).

It is imperative that all possible precautions are taken against possible contamination of the reference material during its production and certification. In order to avoid any contamination, the reference material producer shall identify, preserve and segregate (i.e. from other chemicals and samples) all candidate materials and reference materials, from the time of preparation through to their distribution to users.

The reference material producer shall ensure adequate packaging of all reference materials (e.g. where appropriate, use air-free, moisture-free or inert-gas packaging) and provide secure storage areas/stock rooms which prevent damage or deterioration of any item or material between characterization and distribution. Appropriate methods for authorizing dispatch to, and receipt from, such areas should be stipulated. The condition of all stored/stocked items and materials shall be assessed at appropriate intervals throughout their storage life, in order to detect possible deterioration.

The reference material producer shall be able to demonstrate that the candidate reference material is sufficiently homogeneous; i.e. the difference, if any, between units shall be smaller than the uncertainty limits stated in the certificate.

NOTE A relatively inhomogeneous material may be the best available, and may therefore still be useful as a reference material, provided the uncertainties of the assigned property values take due account of this.

Where appropriate, the property values to be assessed should be measured periodically, ideally over a range of conditions under which the material is to be stored prior to distribution to the user. The effects of light, moisture, heat and time shall be quantified in order to provide advice on storage location and lifespan (and hence a suitable shelf-life/expiry date).

Where appropriate, an assessment of the stability of the assigned property values of the reference material should be performed at periodic intervals after characterization to confirm that all values are maintained from production until its expiry date. Wherever appropriate, the reference material producer shall provide an expiry date for the usable life of the reference materials produced, based on initial and on-going stability studies in compliance with ISO Guide 35.

The reference material producer shall provide details of the homogeneity and stability studies carried out in accordance with the requirements of ISO Guide 31 and ISO Guide 35.

The reference material producer shall issue a statement or certificate, as appropriate, communicating information about the reference material; this shall include information on the property values, their meaning, their uncertainties at a defined confidence level and, where appropriate, the expiry date of the material. The statement or certificate shall also contain information for the user on the proper application of the reference material and on potential problems in its use.

When an expiry date is given the certificate should contain an assurance that the certified value(s) will be monitored at appropriate intervals and that purchasers will be notified of any significant changes resulting in recertification or withdrawal of the CRM during the stated period of validity of the certificate. Even when no expiry date is given and unexpected changes in certified value(s) are detected, purchasers should be informed when these occur within a reasonable period. Producers and distributors shall, therefore maintain a record of purchasers.

See also ISO Guide 31 and ISO Guide 34 [13].

5 Guidance to the user for selection of methods

5.1 General

The specificity of particular food allergens and detection methods may vary considerably. It is therefore important to ensure that the chosen method(s) provide the desired specificity. The following guidance may be useful.

NOTE Most ELISA methods measure protein(s) specific for the allergenic commodity, but not necessarily all relevant allergens. ELISAs detecting one major allergen may not detect other relevant allergens from the same food source. If fragments from one particular allergen are detected by mass spectrometry, other relevant allergens may not be detected as well. These possibilities may be of relevance for interpretation of results obtained on compound food products containing isolated protein fractions derived from allergenic commodities, in particular in case of a negative signal.

Similarly, molecular biology methods detect DNA indicating the presence of an allergenic commodity. In the majority of cases, a positive test result of a molecular biology method will correlate with the presence of the potentially allergenic class of substances, i.e. protein. When purified fractions of a food are used in a compound food product (e.g. processed oils or starch), a positive test result of a molecular biology method may be obtained in the absence of allergenic protein.

5.2 Immunoassay based methods

Proteins can be detected by the application of mono- or polyclonal 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 used antibody needs to be demonstrated (e.g. no cross-reactivity).

Screening methods may be useful to assess whether or not a product is likely to contain allergenic material based on the presence of the expressed protein. Examples of such methods are lateral flow/dip stick format, qualitative ELISAs, biosensors and protein microchips.

Quantitation of the food allergens can be performed by protein based methods such as ELISAs.

The method shall be validated for the matrix to be analysed. Standards shall be available for establishing a standard curve from which calculation of the protein content in test samples is performed.

5.3 Molecular biology based methods

The specificity of analytical methods using DNA as the target to determine the presence of allergenic material depends on the specific properties of the targeted DNA-sequence. Examples of nucleic acid-based methods are PCR, real-time PCR, PCR-ELISA, microarrays and chip technology.

5.4 Chromatographic methods

Chromatography is a separation technique by which proteins, peptides or other markers can be separated from each other based on differences in for instance charge or molecular weight.

Proteins/allergens or markers extracted from the matrix can be injected directly into the chromatographic system, or alternately proteins in the extract are first enzymatically digested to obtain peptides that are then injected into a chromatographic system. The use of mass spectrometry as detection system is preferable, because it can confirm the identity of compounds originating from food allergens based on their typical mass patterns.

6 Laboratory organisation

6.1 General

Compliance with applicable requirements with respect to safety regulations and manufacturers safety recommendations shall be followed according to the guidelines outlined by EN ISO/IEC 17025.

6.2 Laboratory design

Accidental contamination is known to originate from dust and spreading aerosols. As a consequence, the organisation of the work area in the laboratory is logically based on:

a) the systematic containment of the methodological steps involved in the production of the results;

b) a forward flow principle for sample handling.

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

Apparatus and equipment shall be maintained according to manufacturer's instructions.

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

Detailed requirements for the specific methods can be found in EN 15633-1 [6] and EN 15634-1 [7].

7 Procedure

7.1 General

Storage conditions and shelf-life of reagents should be clearly specified. For the use of this standard, general requirements of quality assurance for laboratories shall be observed (e.g. double determination, blanks, use of reference materials, preparation of calibration curves, etc.). Carefully clean all equipment coming into direct contact with the sample to prevent cross contamination. The scope of the method, including applicability to matrices, needs to be clearly defined.

7.2 Preparation of sample

Instructions for the preparation of the laboratory sample should be clearly described in each method.

7.3 Extraction

The appropriate conditions for extraction/dilution of test portions or control and standard materials of known concentration should be described in the instruction for use in detail.

7.4 Preparation of calibration curves

All standards provided with the assay should be used. Matrix matched standards should be used if necessary and available.

7.5 Assay procedure

The assay procedure as given in the protocol shall be followed.

7.6 Quality assurance requirements

Qualitative results from two test portions shall be consistent. If one test portion gives a positive result and the other gives a negative result, then the analysis shall be repeated.

If the quantitative result of one test portion significantly differs from the other the analysis shall be repeated.

8 Interpretation and expression of the results

8.1 General

The parameters to interpret vary depending on whether the assay is qualitative or quantitative. No affirmation shall be made stating that there is no target analyte present in the sample analysed. Negative results shall be reported as "negative at the limit of detection", or "less than the limit of detection". Positive results below the limit of quantification shall be reported as "positive above limit of detection, but below limit of quantification".

8.2 Quantitative analysis

The following parameters are evaluated: raw data (continuous numerical values) of sample test solution, of blank, of reference material or analytical standard, and of negative control, coefficient of variation between replicates, coefficient of variation of standard and coefficient of variation of control samples. All final results should be reported including the measurement uncertainty previously established if required. Quantitative results may not be reported by extrapolating above the highest or below the lowest calibration standard measure.

If the coefficient of variation limit is exceeded, repeat analyses on freshly prepared sample test solution. To establish a coefficient of variation, in this case, at least three determinations shall be carried out (e.g. values from three microtiter wells).

8.3 Qualitative analysis

For qualitative tests, including all applications thereof, the corresponding parameters like sensitivity or specificity are described in the corresponding instructions for use. Results should be reported as detected or not detected and include the limit of detection.

8.4 Provisions

Ideally, the LOD should be provided with reference to the test sample. As a minimum, the LOD shall be provided with reference to a reference material and a relative value based on specified matrices.

For quantitative methods, in addition to LOD, also the LOQ should be reported. However, if the specific target analyte content is below the limit of quantitation, the results shall only be expressed qualitatively.

In the test report the targeted analyte needs to be clearly specified.

8.5 Ambiguous results

The results shall be expressed unambiguously, e.g. not as "+/-". A negative result shall never be expressed as zero value but stated as below the limit of detection of the test.

9 Test report

The test report should follow EN ISO/IEC 17025 guidelines but contain at least the following information:

a) result expressed according to Clause 8;

b) description of the specificity of the analytical method;

c) details of the reference material used.

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