भारतीय मानक
Indian Standard

IS 16579 : 2018
ISO 16578 : 2013

आणविक बायोमार्कर विश्लेषण — माइक्रोएरे द्वारा विशिष्ट न्यूक्लिक एसिड क्रमों की पहचान के लिए सामान्य परिभाषाएँ और आवश्यकताएँ

Molecular Biomarker Analysis — General Definitions and Requirements for Microarray Detection of Specific Nucleic Acid Sequences

ICS 67.050

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Biotechnology for Food and Agriculture Sectional Committee, FAD 23

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 16578 : 2013 'Molecular biomarker analysis — General definitions and requirements for microarray detection of specific nucleic acid sequences' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Biotechnology for Food and Agriculture Sectional Committee and approval of Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.
b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards, which are to be substituted in their places are listed below along with their degree of equivalence for the editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 5725-1 : 1994 Accuracy (trueness and precision) of measurement methods and results — Part 1 : General principles and definitions	IS 15393 (Part 1) : 2003 Accuracy (trueness and precision) of measurement methods and results: Part 1 General principles and definitions	Identical
ISO 5725-2 : 1994 Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method	IS 15393 (Part 2) : 2003 Accuracy (trueness and precision) of measurement methods and results: Part 2 Basic method for the determination of repeatability and reproducibility of a standard measurement method	do
ISO 24276 : 2006 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions	IS/ISO 24276 : 2006 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions	do
ISO/IEC 17025 : 2005 General requirements for the competence of testing and calibration laboratories	IS/ISO/IEC 17025 : 2005 General requirements for the competence of testing and calibration laboratories	do
ISO 22174 : 2005 Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions	IS 16432 : 2015 Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions	do

The technical committee has reviewed the provisions of the following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

International Standard	*Title*
ISO/IEC Guide 99	International vocabulary of metrology — Basic and general concepts and associated terms (VIM)

(*Continued on third cover*)

Indian Standard

MOLECULAR BIOMARKER ANALYSIS — GENERAL DEFINITIONS AND REQUIREMENTS FOR MICROARRAY DETECTION OF SPECIFIC NUCLEIC ACID SEQUENCES

1 Scope

This International Standard defines terms for the detection of nucleic acid sequence of interest using DNA microarrays for detection of nucleic acid.

This International Standard is applicable to all methods that use microarrays for detection of nucleic acids.

This International Standard specifies the verification processes and parameters for molecular biology analysis, including the detection and identification of specific nucleic acid sequences.

This International Standard has been developed to provide recommendations and protocol for

— microarray design and manufacture,

— validation of hybridization specificity,

— interlaboratory validation of qualitative methods,

— determination of limits of detection for a microarray,

— determination of range of reliable signals, and

— criteria to assessing technical performance of the microarray platform.

It does not cover the following protocols:

— the process of quantitative measurement;

— the requirements for sample preparation prior to DNA microarray experiments.

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 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

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

ISO/IEC 17025:2005, *General requirements for the competence of testing and calibration laboratories*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

3 Terms and definitions

For the purposes of this document, the terms and definitions in ISO 5725-1, ISO 5725-2, ISO/IEC 17025, ISO/IEC Guide 99, ISO 22174 and ISO 24276 and the following apply.

3.1
limit of detection for microarray platform
LODP
lowest relative quantity of the external measurement standard (or reference material) that can be detected experimentally at a 95 % confidence level, given a known (determined/estimated) number of copies and/or concentration of the external measurement standard (or reference material)

3.2
range of reliable signal
ability (within a given range) to provide results that are directly proportional to the concentration and/or copy number of the external measurement standard (or reference material)

3.3
DNA microarray
DNA chip
solid substrate where a collection of probe DNA arranged in a specific design is attached in a high-density fashion, directly or indirectly, that assays large amounts of biological material using high-throughput screening methods

3.4
probe DNA
single-strand nucleic acid defined by its property to target specific nucleic acid sequence by base complementarities, where the stringency of the binding is linked with the length and nucleic acid composition of the probes, along with reaction parameters

3.5
platform
device that supports a microarray (or DNA chip) technology

3.6
fluorescence detection
method of detecting hybridization using immobilized probe DNA by measuring a fluorescence signal

3.7
colorimetric detection
method of detecting hybridization using immobilized probe DNA by measuring a colorimetric signal

3.8
electrochemical detection
method of detecting hybridization by measuring electric currents of an electrode onto which probe DNA are immobilized

3.9
external measurement standard
material or substrate prepared for testing the compatibility of the microarray-based methods of analysis, whose property value is derived as a consensus value based on collaborative experimental work under the auspices of a scientific or engineering group

3.10
cross-hybridization
non-specificity binding of probe DNA to non-targeted nucleic acid

4 Principle

4.1 Microarray platform assay

A microarray platform assay, for instance, consists of

— denaturation of the double- or single-stranded DNA or RNA analyte,

— hybridization of the target(s) to probe DNAs bound to a solid substrate,

— detection of hybridization by electrical, colorimetric, and/or fluorescence signals, and

— data analysis.

The laboratory shall implement external measurement standards (or reference material) and suitable controls for the microarray measurement step in the verification process. These requirements governing verification of DNA microarray-based methods also aid in clarifying the interpretations of results.

4.2 Microarray design and manufacture

Microarray analysis should employ the following kinds of probe DNAs, and should be designed to be verifiable.

The design contains probe DNAs for detecting

— external measurement standards (or reference material),

— a positive control,

— a negative control, and

— the nucleic acid sequence of interest.

The immobilized probe DNA shall be replicated at least in duplicate locations. Probe DNAs shall be designed taking into consideration the Tm value, GC ratio, and sequence specificity. The sequence should be described. In order to avoid confusion between nucleotide bases, a lower-case 'g' shall be used to clearly differentiate between 'G' and 'C' in the description (i.e. C, g, A, and T shall be used to indicate bases). The quality of probe DNA shall be ensured by an appropriate method (spectroscopic analysis, mass-spectral analysis, etc.).

4.3 Validation of hybridization specificity

4.3.1 Theoretical assessment of specificity

Theoretical assessment of probe DNA consists of screening one or more of the major nucleic acid sequence databases (such as Refseq: http://www.ncbi.nlm.nih.gov/RefSeq/) with a sequence homology search algorithm (BLAST: http://blast.ncbi.nlm.nih.gov/Blast.cgi, SSEARCH program in FASTA package, etc.). Specific sequences should be selected that are not likely to generate cross-hybridization. These sequences should be tested experimentally.

4.3.2 Experimental assessment of specificity

The specificity of the probe DNAs should be validated experimentally on samples having nucleic acid sequences similar to the target sequence, as well as on organisms identified through the theoretical assessment of specificity as presenting sequence homologies likely to cause cross-hybridizations. The experimental conditions should be the same as those employed routinely by the laboratory.

4.3.3 Experimental assessment of cross-hybridization

The validation process should demonstrate that no cross-hybridizations occur on a probe DNA that is capable of experimentally detecting an external measurement standard (or reference material) in the

matrix. The results are accepted if the probe DNAs for detecting the external measurement standards (or reference material) are all positive and the probe DNAs for detecting negative controls are negative.

4.4 Interlaboratory validation of qualitative methods

4.4.1 General

By their very nature, qualitative tests result only in yes/no answers. However, the determination of the range of use of the method is always necessary in the validation study. The method will only be applicable in that range.

4.4.2 Limit of detection for a microarray platform (LODP)

In the case of microarrays, it is not realistic to determine the limit of detection (LOD) of each target being probed. If LODs of individual targets are required, an external measurement standard (or reference material) could be used for experimental determination of the limit of a series of representative targets on a given platform.

The experimental LODP is related to the test portion, the quality/quantity of the analyte, and the absolute LODP of the method. These values should be established via a collaborative trial using appropriate reference and control samples, and the lowest level of the external measurement standard (or reference material) obtained experimentally should have a false negative rate of less than or equal to 5 %.

4.4.3 Range of reliable signal

The range of reliable signal, i.e. the range of application of the method, should be given for a known (determined/estimated) number of copies and/or concentrations of the external measurement standard (or reference material) at a 95 % confidence level. The values should be established via an interlaboratory trial using appropriate certified reference materials or reference materials. Information may also be derived from intralaboratory studies, as a temporary measure.

4.4.4 Test sample

A solution or an extract containing DNA/RNA molecules appropriate to the field of application is prepared such that there is no demonstrated hybridization inhibition or interference with the electrical, colorimetric, and/or fluorescence detection.

4.4.5 Measuring system

Instruments including thermal cycler, hybridization oven, or other hybridization apparatus, DNA microarray scanner, and apparatus or equipment for measuring DNA/RNA integrity and concentration should be calibrated in accordance with ISO/IEC 17025. This includes criteria for the choice of instrument settings (e.g. background setting, normalization setting).

Any calculations or models used to derive the analytical result should be validated.

4.4.6 Estimation of measurement uncertainty

Uncertainty arises from many sources, including the size of the laboratory sample, sampling of the test sample from the laboratory sample, measurement of the nucleic acid concentration in the extracts, and the sampling of the DNA/RNA in the reactions, as well as the analytical variation.[1] Estimates of the measurement uncertainty may be derived from intralaboratory/interlaboratory study or from estimates of the components, as described by ISO/IEC 17025.

4.4.7 Microarray reagents

The characteristics and quality of reagents [fluorescent dye(s), reverse transcription enzyme, buffers, etc.] and the amount of an external measurement standard (or reference material) that is added to the reaction mixture should be validated.

5 Expression of results

5.1 General

The results shall never be expressed in + and – symbol format.

A negative result shall never be expressed in the "absence of target sequence" format.

5.2 Expression of a negative result

The following sentences or equivalent shall appear in the test report.

The DNA/RNA (specify) target sequence Y was not detected.

The LODP of the method was X determined with ABC (describe the external reference materials).

5.3 Expression of a positive result

The following sentences or equivalent shall appear in the test report.

The DNA/RNA (specify) target sequence Y was detected.

The identity of the target(s) may be included, if known.

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

5.4 Expression of an inconclusive result

A validated method includes criteria from which an observed measurement result can be accepted as valid. The accept/reject criteria for the analysis shall be described.

The test report shall include information on the repeatability standard deviation and reproducibility standard deviation.

When at least one test portion gives inconclusive results, then the analyses shall be repeated.

If repetition of the analysis verifies the inconclusive result, the test report shall feature the following information:

— "inconclusive result";

— the reason a conclusive result could not be obtained (e.g. inhibition effects, interfering substances, etc.).

6 Test report

Reporting should be carried out as specified in applicable standards (e.g. ISO/IEC 17025).

The test report shall include at least the following information:

— all information needed to identify the sample;

— the date of receipt;

— any particular information relating to the laboratory samples and any concomitant restrictions that may apply;

— all information related to the test sample (the type, number of the sample);

— any conditions regarding sample transport, as well as all storage, if applicable;

— statement about the date and the type of sampling procedure(s) used, if applicable;

— a description of the nucleic acid extraction method used;

— identification of the analysis method and a general description of the microarray process that forms the basis of the analytical method(s);

— both the positive and negative controls;

— the type of external measurement standards (or reference materials);

— the results of experimental LODP and the range of reliable signal for detection analyses;

— any calculations or models used to derive the analytical result;

— any outstanding points observed during testing.

Bibliography

[1] ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*

(*Continued from second cover*)

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 2016 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

This Indian Standard has been developed from Doc No.: FAD 23 (2767).

Amendments Issued Since Publication

Amendment No.	Date of Issue	Text Affected

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