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Molecular biomarker analysis — General definitions and requirements for microarray detection of specific nucleic acid sequences

Analyse moléculaire des biomarqueurs — Définitions générales et exigences relatives à la détection sur microréseaux de séquences d'acides nucléiques spécifiques

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

Introduction

The main focus of this International Standard is DNA microarray-based methodologies.

DNA microarray is a molecular biological technique capable of simultaneous detection of multiple nucleic acid sequences and is particularly suitable for identifying nucleic acid sequences of interest and for measuring gene expression levels. Microarray and its derived technology have been developed for use in the field of food analysis [genetically modified organism (GMO) analysis, biomarker identification, etc.]. Although the standardized parameters required for DNA microarray-based methods have been under consideration (such as MAQC and MIAME), it is necessary to generate minimum requirements for the interpretation of the results.

Therefore, the aim of this International Standard is to provide guidance and requirements for the detection of nucleic acid sequences of interest by microarrays. This information concerns

— the establishment of validation approaches for methods based on DNA microarray, and

— the definition of general principles employed in carrying out these laboratory analyses.

Microarray technology is evolved from Southern blotting; the core principle is hybridization between two DNA strands, by the property of complementarities of nucleic acid sequences. A DNA microarray is a collection of microscopic DNA spots attached to a solid substrate or coded beads. The development of a microarray assay generally needs to design the probe DNAs, to arrange the probe DNAs onto a solid substrate, to label the target nucleic acid sequences, to hybridize the targets with the probe DNAs, and to elaborate an appropriate detection system. Many types of arrays exist and there are many ways to fabricate microarrays. According to the target labelling techniques used, the hybridization can be detected by electrical, colorimetric, and/or fluorescence signals.

At the time of publication of the International Standard, best practices and standards for data representation and minimum information have been developed for comparability and reproducibility of microarray data. However, only few published work have yet focused on the reliability and comparability of any given microarray platforms, and a single-laboratory validation would most likely not suffice in this case. Rather, an interlaboratory method validation should be adopted, according to specific international guidelines.

NOTE 1 The Microarray Quality Control (MAQC) consortium provides an excellent resource to determine best microarray practices, including the use of reference material, data assembly, and formats.

See http://www.fda.gov/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/default.htm

NOTE 2 The Minimum Information About a Microarray Experiment (MIAME), by establishing common standards for describing microarray data, systems for data management and transfer, and public repositories for data storage and mining, aids in describing the detailed information that researchers should provide to explain the procedures and biological purposes of their microarray data.

See http://www.mged.org/Workgroups/MIAME/miame.html

General requirements for the detection of DNA are also laid down in the following International Standards: ISO 21569, ISO 21570, ISO 21571, ISO 22174 and ISO 24276.

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;