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In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids —

Part 3: Interpretation and reports

Dispositifs médicaux de diagnostic in vitro — Tests moléculaires multiplex pour les acides nucléiques —

Partie 3: Interprétation et rapports

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Contents

Page

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 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 212, *Medical laboratories and in vitro diagnostic systems.*

A list of all parts in the ISO 21474 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The first generation of in vitro diagnostic (IVD) medical devices for nucleic acid-based molecular tests has been focused on detection or quantitation of a single nucleic acid sequence (e.g. viral RNA, mRNA, or genomic DNA) within a clinical specimen. By comparison, a multiplex molecular test simultaneously measures multiple nucleic acid sequences of interest in a single reaction tube or a system. The development and clinical use of multiplex IVD medical devices are rapidly expanding with the technological advances and new elucidation of the clinical significance of many biomarkers.

In comparison to single target analysis, multiplex molecular tests require an increased number of controls, more complex performance evaluation/data analysis algorithms, and more complex interpretation and reporting of results.[1,2] Some multiplex systems amplify multiple targets in a single reaction step and then split these into reactions for specific target detection.[3]

Laboratories can develop assays in-house ("laboratory-developed test (LDT)", "home-brew", or "in-house test") or use commercially available multiplex assays involving a variety of technologies and instrument platforms. Multiplex molecular testing provides large amounts of complicated and multifarious genetic information, resulting in significant challenges to the laboratory with regards to appropriate data analysis, interpretation and reporting.

Implementation of a multiplex molecular test identifies large numbers of genetic variations in a sample, which is crucial for optimal patient care, and treatment guidelines are developed based on specific molecular findings; therefore, it is imperative to standardize the interpretation and reporting of molecular results among laboratories performing these tests.

This document describes the requirements and recommendations for various aspects of interpretation and reporting of the results by multiplex molecular tests in order to ensure the quality of laboratory services of such tests, in implementing multiplex molecular nucleic acid tests for clinical use.

In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids —

Part 3: Interpretation and reports

1 Scope

This document gives the general requirements for interpretation and reporting of multiplex molecular tests which simultaneously identify two or more nucleic acid target sequences of interest. This document is applicable to all multiplex methods used for examination using in vitro diagnostic (IVD) medical devices and laboratory developed tests (LDTs). It provides information for both qualitative and quantitative detection of nucleic acid target sequences.

This document is intended as guidance for multiplex examinations that detect or quantify human nucleic acid target sequences and microbial pathogen nucleic acid target sequences from human clinical specimens.

This document is applicable to any molecular IVD examination performed by medical laboratories. It is also intended to be used by laboratory customers, IVD developers and manufacturers, biobanks, institutions, commercial organizations performing biomedical research, and regulatory authorities. This document is not applicable to metagenomic massive parallel sequencing (MPS), but it is applicable to multiplex molecular methods including 16S sequencing.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO 21474-1, *In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids — Part 1: Terminology and general requirements for nucleic acid quality evaluation*

3 Terms and definitions

For the purposes of this document, terms and definitions given in ISO 21474-1 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
process step
part of a process which is predominantly self-sufficient and consists of one or several unit operations

[SOURCE: ISO 10209:2022, 3.1.65]

4 General requirements

Multiplex molecular tests are IVD and medical devices that measure multiple nucleic acid sequences simultaneously, such as multiplex PCR, DNA microarray, and MPS-based methodologies.

A multivariable molecular test is a molecular test that combines the values of multiple variables using an interpretation function to yield a single patient-specific result including “classification”, “score” and/or “index”. This is usually based on a platform of multiplex molecular tests, e.g. a miRNA assay.[5] For more guidance, see Annex B.

An increasing number of clinical and commercial laboratories have been performing multiplex molecular tests and issuing corresponding clinical reports to provide information for the care of their patients. However, the detected variants and relevant information in each report can differ because of the use of different methodologies (e.g. multiplex PCR, DNA microarray, and MPS-based), panels (e.g. commercial panels or laboratory-developed test panels), target enrichment strategies (e.g. targeted capture or multiplex PCR), sequencing platforms, improvement countermeasures, bioinformatics analysis processes, and databases (e.g. public databases or self-built databases) by different laboratories.

Based on accurate results of testing, laboratories shall make evidence-based testing interpretation and release accurate and comprehensive reports, to ensure the best diagnosis and treatment strategies for patients.

NOTE Further guidance on MPS is given in ISO 20397-2.

5 Interpretation of results

5.1 General

The interpretation method shall be fit for purpose and should be supported by a relevant validation study.

The laboratory shall have documented procedures for interpretation and reporting of results, including algorithms, software, and databases. Procedures for interpretation shall include measures to minimize the risk of cognitive bias.

For the implementation of quality management in the process of interpretation, see ISO/IEC/IEEE 90003, which provides guidance for organizations in the application of ISO 9001 to the acquisition, supply, development, operation and maintenance of computer software, and related support services.

Results by multiplex molecular tests, e.g. detected variants and combined values of multiple variables, should be carefully reviewed by appropriately trained molecular diagnostic professionals in the context of each complete case, including histological and clinical findings.

Evidence-based categorization, e.g. “classification”, “scoring” and/or “indexing”, shall be performed before reporting. Genomics is a rapidly evolving field; therefore, the clinical significance of any variant in therapy, diagnosis, or prognosis should be re-evaluated on an ongoing basis.

5.2 Methods for interpretation of results

Methods to analyse the test results can differ, depending on the intended use of the test and whether the test results are qualitative or quantitative in nature.

Multiplex molecular tests, such as DNA microarray, and MPS-based methodologies comprise wet analysis and bioinformatic processes. Bioinformatic processes should include considerations on genomic databases, reference sequence databases, variant identification annotation and categorization, and curation.

In interpretation of results of multiplex molecular tests, the laboratory should take it into consideration that the positive predictive value (PPV) and negative predictive value (NPV) of each target of detection is influenced by the prevalence of target diseases or conditions of interest.

A multivariate assay with algorithmic analyses combines the results of two or more biomarkers, with or without patient demographics and clinical information, into an algorithm to generate a classifier to stratify patients into different outcome groups for subsequent clinical follow-up. The algorithm can be a simple linear regression model or more complicated non-linear model(s) when required.

Where a cut-off is applicable to an assay, the cut-off should be used to determine the clinical sensitivity and clinical specificity.

When the multi-variate assays, such as miRNA-based assays, are generated from multiple analytes with no diagnostic value for the individual analytes, the result should be described using the risk scores instead of using the reading from the individual analytes.

In case of multivariable molecular test (e.g. miRNA analysis), the algorithm integrates the expression levels of analytes and normalizes it into a single numerical score that classifies the individuals into positive, negative and in some cases, intermediate outcome groups. Since the inputs to algorithm are an individual analyte expression level or concentration, the validity of the algorithm shall be monitored.[2],[5] For more guidance, see Annex B.

As manual interpretation is prone to missing critical information generated by multiplex molecular testing, laboratories should put in place an automated procedure for the process of interpretation based on updating informative databases in a timely manner.

5.3 Documentation on bioinformatics analysis

The laboratory shall use a documented standard operating procedure (SOP) for bioinformatics to analyse, interpret, and report the results. A complete procedure manual shall be available on the workbench or in the work area.

The laboratory shall document all algorithms, software, and databases used in the analysis, interpretation, and reporting of results.

The versions of each of these components in the overall bioinformatics shall be recorded and traceable for each patient result.

For each component, the laboratory can use a baseline, default installation, or it can customize the process by using alternate configuration parameters in deploying individual bioinformatics tools or in running specific algorithms. These customized tools should be adopted to the extent that they do not affect the performance of the test and may be subject to additional verification and validation steps.

The laboratory shall document any customizations that vary from the specified configuration, namely which parameters, cut-offs, and values are used.

When describing the bioinformatics process, the laboratory should document the overall workflow of the data analysis and include the input and output files for each process step. For each step, the laboratory should develop and document acceptable quality control parameters for ensuring the specified performance characteristics.

Where applicable, the laboratory should develop and document criteria for variant calling and called parameters, including thresholds for read coverage depth, variant quality scores, and allelic read percentages.

Evidence of compliance with this document, i.e. ISO 21474-3, should be demonstrated with appropriate documentation.

The laboratory should also document the bioinformatics processes that are used for reducing a large data set to a list of either causal relation or candidate genes or variants or both. For example, in inherited disease assays, the laboratory should document approaches used to identify recessive (latent or occult), dominant (overt or explicit), and new variants.

Where applicable, bioinformatics analyses are conducted by aligning sequence reads to a reference sequence. The reference sequence version number and assembly details shall also be identified. Further information is available in ISO 20397-2.

Variants shall be named according to international nomenclature used by sector organizations standards (e.g. The Human Genome Variation Society (HGVS), the Internal System for Human Cytogenetic Nomenclature (ISCN), and the International Union of Microbiological Societies)[1)], allowing explicit mapping to standardized reference numbers.

As the number of targets of interest increases in a multiplex assay, false negative (FN) results for certain sequences can become more problematic. In particular, the target with the lowest abundance within the nucleic acid sample should be assessed.

As the number of targets of interest increases, false positive (FP) results can become more problematic. For example, intrinsic limitation of microarrays is probe cross-hybridization to similar sequences within a genome. Sequence errors can also occur during nucleic acid amplification, leading to incorrect base calling. There is also a risk of FP results due to contamination while collecting and handling clinical specimens. Thus, the influence should be assessed with an appropriate method, such as using the quantitative measurement with cut-off values.

5.4 Monitoring of bioinformatics analysis

For bioinformatic analysis of generated data, the laboratory shall monitor validated performance of parameters, including robustness, accuracy, and reproducibility at each step.

5.5 Genomic databases

The genomic databases provide information that is necessary for accurate annotation and prioritization of variants. Laboratories should exercise the following cautionary steps on the use of public databases:

a) Understand the content of the database and how the data are aggregated. The laboratory should review the documentation or published literature relating to a given database to ascertain the source, type, and intent of the database.

b) Pay specific attention to the limitation of each database to avoid overinterpretation of annotation results.

c) Confirm the versions of the reference sequence version and assembly details as well as mRNA transcript references to ensure appropriate HGVS annotation or ISCN.

d) Whenever possible, use genomic coordinates, instead of HGVS nomenclature or ISCN, to unambiguously query genomic databases.

e) Assess the quality of the provided genomic data based on the source, from publications or another database, the number of a specific entries (single or multiple), the depth of the study, the use of appropriate controls, confirmation of a variant's somatic origin, and functional and potential drug response studies.

f) Verify data quality of the pathological diagnosis when provided (e.g. site, diagnosis, and subtype).

1) The Human Genome Variation Society (HGVS)
https://hgvs-nomenclature.org/stable/ https://hgvs-nomenclature.org/stable/
The Internal System for Human Cytogenetic Nomenclature (ISCN)
https://iscn.karger.com
The International Union of Microbiological Societies
https://www.the-icsp.org/index.php/international-union-of-microbiological-societies