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

Part 1: Terminology and general requirements for nucleic acid quality evaluation

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

Partie 1: Terminologie et exigences générales pour l'évaluation de la qualité des acides nucléiques

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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 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test 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 diagnostics (IVD) medical devices for nucleic acid-based molecular tests have 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. The development and clinical use of multiplex IVD medical devices are rapidly expanding with technological advances and new elucidation of the clinical significance of many biomarkers.

The measurement of multiple analytes of interest in a clinical specimen is generally performed by the following successive (or simultaneous) steps. After specimen collection, transport and storage, nucleic acids are extracted, with or without a subsequent purification procedure. The nucleic acid is then quantified, and its quality evaluated (if necessary), diluted (if necessary) and subjected to multiplex molecular test(s). Multiplex molecular tests in current clinical use detect DNA or RNA targets using various techniques, such as multiplex PCR examinations, microarrays, mass array or massive parallel sequencing-based methodologies.

Although quality aspects of nucleic acids for single target molecular analysis (such as singleplex PCR) has been described[1][2], this cannot necessarily be applied to multiplex molecular tests. Due to the inherent competition for more than one nucleic acid target in a multiplex assay, these assays are usually more sensitive to the isolated nucleic acid quality and quantity than single target assays. The variability of each specimen in biological, physical and chemical properties can influence the performance of multiplex assays to a larger degree than single target assays, potentially leading to unreliable results and hampering patient care. Thus, sample quality evaluation should require additional considerations for multiplex molecular tests.

The collection, transport and preparation of specimens for medical laboratory use has been addressed in national and international efforts in general including ISO/TS 20658 “Medical laboratories—Requirements for collection, transport, receipt and handling of samples”[3], “Guideline for the Quality Management of Specimens for Molecular Methods, The Procurement, Transport, and Preparation of Specimens” (Japan, JCCLS)[4] and “Guideline for the Quality Management of Specimens for Molecular Methods (Part 2) New Technologies and Sample Quality Control (Japan, JCCLS)”[5], and more specifically for different biological specimen types in the series of ISO 20166, 20184, and 20186[6][7][8].

This document describes the terminology and general quality requirements for nucleic acid used in multiplex molecular tests, in order to ensure reproducible performance of such tests.

NOTE Guidelines, requirements, and performance criteria laid down in this document, are intended to ensure that comparable, accurate and reproducible results are obtained in different laboratories.

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

Part 1: Terminology and general requirements for nucleic acid quality evaluation

1 Scope

This document provides the terms and general requirements for the evaluation of the quality of nucleic acids as the analytes for multiplex molecular tests, which simultaneously identify two or more nucleic acid target sequences of interest. This document is applicable to all multiplex molecular 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 molecular assays that detect and/or quantify human nucleic acid target sequences or microbial pathogen nucleic acid target sequences from human clinical specimens. This document is applicable to any molecular in vitro diagnostic examination performed by medical laboratories. It is also intended to be used by laboratory customers, in vitro diagnostics developers and manufacturers, biobanks, institutions and commercial organizations performing biomedical research, and regulatory authorities. This document is not applicable to metagenomics.

NOTE An examination procedure developed for a laboratory's own use is often referred to as a "laboratory developed test", "LDT", or "in-house test".

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 15189:2012, *Medical laboratories — Requirements for quality and competence*

3 Terms and definitions

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

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

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

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

3.1
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurand

Note 1 to entry: The term accuracy, when applied to a set of test results, involves a combination of random components and a common systematic error or bias component (ISO 3534-2:2006, 3.3.1).

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — "NOTE 1", "NOTE 2" and "NOTE 3" have been deleted, and new "Note 1 to entry" has been added.]

3.2
algorithm
a set of rules or calculations applied to test data that generate an interpretable or reportable result

3.3
allele
<genetics> any of several forms of a gene that is responsible for hereditary variation

Note 1 to entry: An allele can also be defined as:

Note 2 to entry: 1) one of the alternate forms of a polymorphic DNA sequence that is not necessarily contained within a gene;

Note 3 to entry: 2) one of the alternative forms of a gene that may occupy a given locus.

3.4
allelic ratio
the ratio of a specified *allele* (3.3) to the total number of *alleles* (3.3), normally expressed as a fraction

Note 1 to entry: For example, if a specific *allele* (3.3) represents 40 % of the total *alleles* (3.3) found at a given locus, the allelic ratio is 0,4.

Note 2 to entry: Allelic ratio is synonymous with allele frequency.

3.5
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2, modified — The example has been deleted.]

3.6
chemical purity
degree of contamination with chemical substances that influences the multiplex analysis

Note 1 to entry: The purity of nucleic acid for PCR is absence of interfering organic and protein components carried through from the extraction step, as well as contaminating nucleic acids.

3.7
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

[SOURCE: ISO 16578: 2013, 3.3]

3.8
documented procedure
specified way to carry out an activity or a process that is documented, implemented and maintained *interlaboratory comparison* (3.12)

3.9
evaluation method
method of evaluating the quality specified for nucleic acid

3.10
expiry date
expiration date
upper limit of the time interval during which the performance characteristics of a material stored under specified conditions can be assured

Note 1 to entry: Expiry dates are assigned to *IVD reagents* (3.15), calibrators, control materials and other components by the manufacturer based on experimentally determined *stability* (3.35) properties.

[SOURCE: ISO 18113-1:2009, 3.17, modified — "Note 2 to entry" and "Note 3 to entry" have been deleted.]

3.11
external measurement standard
reference standard
material or substrate prepared for testing the compatibility of the methods of multiplex analysis, whose property value is derived as a consensus value based on collaborative experimental work under the auspices of a scientific or engineering group

Note 1 to entry: This is commonly targeted at the multiplex molecular analysis.

Note 2 to entry: Reference material can be used as an alternative of external measurement standard.

[SOURCE: ISO 16578:2013, 3.9, modified — "Note 1 to entry" and "Note 2 to entry" have been added.]

3.12
intended use
intended purpose
objective intent of an IVD manufacturer regarding the use of a product, process or service as reflected in the specifications, instructions and information supplied by the IVD manufacturer

[SOURCE: ISO 18113-1:2009, 3.31, modified — "Note 1 to entry" and "Note 2 to entry" have been deleted.]

3.13
interlaboratory comparison
organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions

[SOURCE: ISO/IEC 17043:2010, 3.4]

3.14
in vitro diagnostic instrument
IVD instrument
equipment or apparatus intended by a manufacturer to be used as an *IVD medical device* (3.14)

[SOURCE: ISO 18113-1:2009, 3.26, modified — "Note 1 to entry" has been deleted.]

3.15
in vitro diagnostic product
in vitro diagnostic medical device
IVD medical device
reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae

[SOURCE: 21CFR809.3 of the US Federal Food, Drug and Cosmetic Act]

3.16
in vitro diagnostic reagent
IVD reagent
chemical, biological, or immunological components, solutions or preparations intended by the manufacturer to be used with an *IVD medical device* (3.14)

[SOURCE: ISO 18113-1:2009, 3.28, modified — "Note 1 to entry" has been deleted.]

3.17
laboratory developed tests
LDTs
type of in vitro diagnostic devices that are intended for clinical use and are designed, manufactured and used within a single laboratory

Note 1 to entry: It is often referred to as a "in-house test".

[SOURCE: CLSI QSRLDT]

3.18
limit of detection
LOD
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

Note 2 to entry: This is for LODs when the tests are evaluating the presence or absence of multiple *analytes* (3.5) rather than a *multivariable molecular test* (3.25).

Note 3 to entry: Limit of detection, LOD, is alternatively defined as 1) the lowest quantity of a nucleic acid that can be sequenced reliably and distinguished from its absence typically within a stated confidence limit; 2) the minimum detectable allelic fraction in a given sample.

[SOURCE: CLSI MM09 2014]

3.19
limit of detection for microarray platform
limit of detection for multiplex molecular test platform
LODP
lowest relative quantity of the *external measurement standard* (3.10) (or reference material) that can be consistently detected experimentally at a 95 % confidence level, given a known (determined/estimated) number of copies and/or concentration of the *external measurement standard* (3.10) (or reference material)

Note 1 to entry: This is commonly targeted at the multiplex molecular analysis.

Note 2 to entry: LODP can be used as a performance indicator replaced by *limit of detection* (3.18) for multiplex analysis.

[SOURCE: ISO 16578:2013, 3.1, modified — "Note 1 to entry" and "Note 2 to entry" have been added.]

3.20
massive parallel sequencing
methodology that enables high-throughput DNA sequencing using the concept of processing a very large number of molecules in parallel

Note 1 to entry: For example but not limited to the technologies with miniaturized and parallelized platforms for sequencing of thousands to millions of short reads (≈50 to 400 bases), or polymerase-based real-time DNA sequencing platform enabling long read (mean length ≈10,000–15,000 bases).

3.21
microRNA
17 to 25 nucleotide-long single strand RNA relating to post transcriptional expression regulation

3.22
multiple sequences of analyte(s)
constituent of a sample with multiple sequences of nucleic acid measured simultaneously

Note 1 to entry: This includes extracted nucleic acid and that before and/or after amplification in case of nucleic acid amplification-based assay.

3.23
multiplex molecular test
in vitro diagnostic test that simultaneously evaluates sequence identity and/or amounts of multiple, namely two or more nucleic acid targets of interest in a single run of the assay, such as *multiplex PCR* (3.24), multiple hybridization detection, microarray and *massive parallel sequencing* (3.19) based methodologies

Note 1 to entry: “Multiplex” is defined as “those in which two or more targets are simultaneously detected through a common process of sample preparation, target or signal amplification, *allele* (3.3) discrimination, and collective interpretation. (CLSI/MM17-A[12]).

Note 2 to entry: Targets of interest is defined as detection targets of interest and exclude the control material from being a target.

3.24
multiplex molecular test quality nucleic acid
nucleic acid template with appropriate property that ensures the measurement by a *multiplex molecular test* (3.22) such as that of sufficient length, quantity, *chemical purity* (3.6), *structural integrity* (3.36), and presence of nucleic acid sequence of interest

3.25
multiplex PCR
PCR technique that employs multiple pairs of primers combined within a single reaction mixture to produce multiple amplicons simultaneously

[SOURCE: ISO 16577:2016, 3.117]

3.26
multivariable molecular test
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”

Note 1 to entry: This is usually based on a platform of multiplex molecular tests.

Note 2 to entry: This is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease.

Note 3 to entry: The term “multivariable” as used in statistics implies the evaluation of multiple outcomes rather than using multiple variables to evaluate a single outcome.

3.27
pathogen
infectious agent that causes diseases in its host

Note 1 to entry: Pathogen includes some virus, viroid, prion, bacterium, fungus, or parasite.

[SOURCE: ISO 15714:2019, 3.1.2, modified.]

3.28
PCR quality DNA
DNA template of sufficient length, quantity, *chemical purity* (3.6), and *structural integrity* (3.36) to be amplified by PCR

[SOURCE: ISO 24276:2006, 3.2.3, modified — “quantity” is added.]