



International
Standard

ISO 21474-3

**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

**First edition
2024-11**

Reference number
ISO 21474-3:2024(en)

Full document details: ISO 21474-3-2024

© ISO 2024  **Documents**
Document Sharing Hub



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 General requirements	2
5 Interpretation of results	2
5.1 General	2
5.2 Methods for interpretation of results	2
5.3 Documentation on bioinformatics analysis	3
5.4 Monitoring of bioinformatics analysis	4
5.5 Genomic databases	4
5.6 Reference sequence databases	5
5.7 Variant identification and annotation	5
5.8 Categorization of variants	6
6 Reporting of test results	7
6.1 General	7
6.2 Reporting elements	7
6.3 Test report content	8
6.4 Reporting detected variants	9
6.5 Reporting of secondary findings	9
6.6 Reporting method	10
Annex A (informative) Multiplex molecular test for cancer	11
Annex B (informative) Multivariable molecular tests	12
Annex C (informative) Chromosome microarray analysis test reports	13
Bibliography	14

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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]