
**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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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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Foreword

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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.

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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.