CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

MM09

Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods

This guideline, in conjunction with instructional worksheets and educational examples, provides step-by-step recommendations for design, development, validation, results reporting, and continual quality management of clinical tests based on next-generation sequencing and Sanger sequencing.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Abstract

Sequencing-based clinical tests have evolved from single-gene tests to whole-genome tests. Next-generation sequencing (NGS) technologies have largely replaced Sanger sequencing and are firmly established in the medical management of hereditary disorders, as well as in tumor testing. Newer clinical NGS applications include human leukocyte antigen typing, noninvasive prenatal testing, sequencing of circulating tumor DNA in peripheral blood, and RNA sequencing. Although NGS applications have undergone major technical simplifications, clinical implementation continues to be complex. Clinical and Laboratory Standards Institute guideline MM09—*Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods* provides recommendations for design, development, validation, results reporting, and continual quality management of NGS-based tests, as well as Sanger sequencing–based tests. In conjunction with instructional worksheets and educational examples, MM09 provides step-by-step guidance to help medical laboratories translate regulatory requirements into clinical practice.

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Foreword

Sequencing-based clinical tests have existed for three decades, evolving from the single-gene tests used in the late 1980s to the whole-genome tests currently in use. The introduction of next-generation sequencing (NGS) catalyzed this evolution. Increasingly, NGS is replacing Sanger sequencing, particularly when examining a large number of genes is critical for maximum clinical utility. Today, NGS is firmly established in the medical management of hereditary disorders, especially those with clinical and genetic heterogeneity, as well as in tumor testing (ie, somatic NGS). Laboratory and medical practices for these clinical applications are relatively mature, and guidance from several professional societies and other expert groups is available (see Appendix A).

More recently, NGS has been used in additional areas of clinical practice, including human leukocyte antigen typing and noninvasive prenatal testing of fetal DNA in maternal blood to detect the presence or absence of select pathogenic variants in the fetus. Furthermore, new approaches provide additional opportunities for use in clinical areas in which NGS-based testing is already established. Innovative applications include RNA-based NGS (ie, RNA sequencing) to detect gene fusions and liquid biopsy (ie, DNA-based NGS) to detect genetic alterations in circulating tumor DNA in peripheral blood.

Although NGS applications have undergone major technical simplifications, clinical implementation continues to be challenging. Additional guidance is needed to ensure the technical and clinical validity of NGS tests. Guidance is increasingly important because genomic testing is being commoditized, and test developers vary in their interpretation and implementation of existing regulatory frameworks.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM09-A2, published in 2014. MM09-A2 introduced NGS as a new technology. This edition has been updated beyond an introduction of NGS technology to provide practical use case and implementation guidance, as well as instructions that cover each step of the clinical NGS test development lifecycle. Several changes were made in this edition, including:

- Providing step-by-step recommendations on designing, developing, validating, and implementing a clinical NGS test
- Adding clear and specific instructions on performing steps in the clinical NGS test lifecycle
- Presenting an application-driven approach
- Providing educational use case examples, supplemented by instructional worksheets
- Updating appendixes with additional details on steps in the test development process and specific applications

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

bioinformatics
design
development
germline
human leukocyte antigen
implementation
liquid biopsy
next-generation sequencing
noninvasive prenatal testing
optimization
quality management
RNA sequencing
somatic
validation
verification

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Chapter 1

Introduction

Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods

1 Introduction

1.1 Scope

This guideline covers nucleic acid sequencing applications currently in clinical use: medical management of hereditary disorders, solid tumor and hematological malignancy testing, human leukocyte antigen (HLA) typing, noninvasive prenatal testing (NIPT), liquid biopsy, and RNA sequencing (RNAseq) applications. Most of the content in this guideline focuses on next-generation sequencing (NGS), which is the predominant platform in current use. Sanger sequencing continues to be used for certain clinical applications, so guidance on Sanger sequencing is also included. This guideline also provides introductory information on the management of computational and/or bioinformatics aspects of NGS, because these concepts are fundamental yet somewhat novel for the clinical testing community. Detailed guidance on bioinformatics will be provided in a forthcoming CLSI document.

MM09 does not cover microbial or infectious diseases applications. Detailed guidance on NGS-based infectious diseases testing is provided in CLSI document MM24.[1] This guideline also does not cover validation of confirmatory testing or mitochondrial DNA testing for inherited disorders.

This guideline is intended for developers of sequencing-based clinical tests (both Sanger sequencing and NGS), including manufacturers of commercially distributed *in vitro* diagnostic (IVD) devices and developers of laboratory-developed tests (LDTs). IVD device manufacturers might be subject to additional quality system requirements. For example, design controls are not included in this guideline, but they are well described in existing literature.[2,3]

1.2 Background

MM09 provides step-by-step guidance on development of clinical sequencing tests. Topics include test familiarization, design, development, and optimization, as well as analytical validation and quality management. This guideline specifically focuses on explaining **how to implement** sequencing technologies in a clinical setting (ie, how to develop and analytically validate sequencing-based clinical tests) rather than providing in-depth education on **how they work,** because a large body of literature covers the latter. **NOTE:** This guideline refers to US Food and Drug Administration (FDA) requirements. FDA requirements do not apply to test developers outside the United States.

MM09 contains:

- Traditional, text-based chapters that outline the clinical test development process and provide a high-level introduction, background information, and necessary context for the test developer
- A link to instructional worksheets (shared resources with the College of American Pathologists) that provide additional information and concrete guidance, including forms adaptable by the user and educational examples (see Additional Resources)
- Appendixes with additional resources and detailed information, including descriptions of technology platforms