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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Contents

Abstract ii
Committee Membership iii
Foreword vii
Chapter 1: Introduction 1
1.1 Scope 2
1.2 Background 2
1.3 Standard Precautions 3
1.4 Terminology 3
Chapter 2: Next-Generation Sequencing Test Development Lifecycle 15
Chapter 3: Application-Agnostic Considerations 19
3.1 Test Familiarization 20
3.2 Test Design, Development, and Optimization 21
3.3 General Paradigms for Analytical Test Validation, Revalidation, and Verification 22
3.4 Results Confirmation 26
3.5 Quality Management System 27
3.6 Bioinformatics Infrastructure Considerations 29
Chapter 4: Clinical Applications of Sequencing 41
4.1 Sanger Sequencing 42
4.2 Next-Generation Sequencing for Hereditary Disorders and Tumors 43
4.3 Human Leukocyte Antigen Sequencing 70
4.4 Circulating DNA Applications: Noninvasive Prenatal Testing and Liquid Biopsy 81
4.5 RNA Sequencing 92
Chapter 5: Conclusion 101
Chapter 6: Supplemental Information 103
References 104
Additional Resources 119
Appendix A. Next-Generation Sequencing General Recommendations and Guidelines 120
Appendix B. Test Design and Optimization Additional Details 124
Appendix C. Somatic Applications Examples 132
Appendix D. Interpretation and Reporting Additional Details 134
The Quality Management System Approach 148

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

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

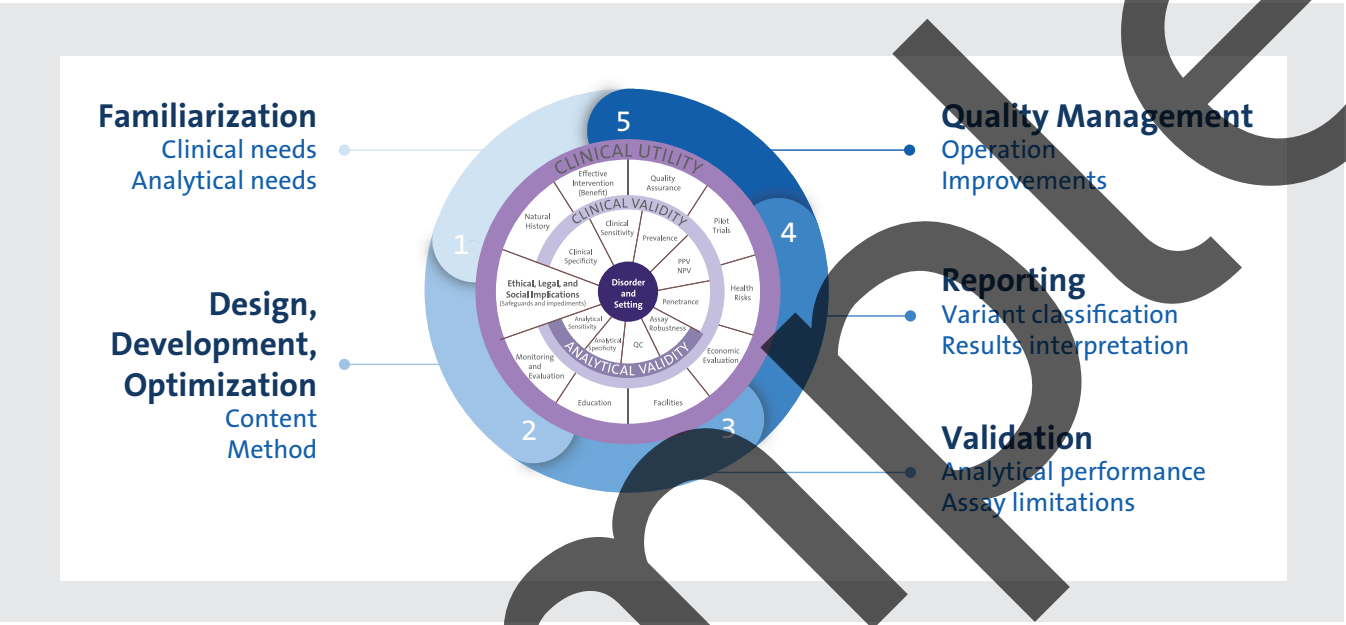
MM09 provides step-by-step guidance on development of clinical sequencing tests. Topics include test familiarization, design, development, and optimization, as well as analytical validation and quality management. This guideline specifically focuses on explaining **how to implement** sequencing technologies 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

2 Next-Generation Sequencing Test Development Lifecycle

MM09 is structured to follow the steps in the NGS clinical test development process, from general familiarization to test design to operation (see Figures 1A and 1B). Current clinical sequencing applications share common elements, but they can diverge significantly depending on the application. This guideline minimizes redundancy by separating application-agnostic content (in Chapter 3) from application-specific content (in Chapter 4). Content is combined when most requirements for applications are identical (eg, germline and somatic sequencing). For germline and somatic sequencing, additional and diverging requirements for each application are clearly indicated.



Abbreviations: NPV, negative predictive value; PPV, positive predictive value; QC, quality control.

Figure 1A. NGS Test Development Lifecycle[23] (Courtesy of Centers for Disease Control and Prevention [CDC], Genomics & Precision Health. ACCE Model Process for Evaluating Genetic Tests. CLSI's use of the material, which is freely available on the CDC website, does not imply endorsement by CDC or the US government of CLSI or its products.)

4 Clinical Applications of Sequencing

Clinical applications of sequencing technologies are rapidly evolving, and sequencing test results are increasingly used in medical decision-making. DNA sequencing is predominantly used across clinical areas, and NGS technologies have largely replaced Sanger sequencing. NGS applications include gene panels and, increasingly, exome and genome sequencing. Compared with older sequencing technologies, NGS provides increased throughput and scalability. Additionally, as more genes and/or variants that cause diseases are identified, NGS readily enables content modifications for diagnostic exome and/or genome applications. However, Sanger sequencing is still the preferred method for certain clinical applications and is expected to be integral to clinical molecular testing for years to come. Table 4 lists the clinical applications and technical approaches covered in this guideline.

Table 4. Clinical Applications and Technologies Covered in MM09

Clinical Applications	Technical Approaches			
	Sanger Sequencing	DNA-based NGS	RNA-based NGS	Circulating cfDNA by NGS
Inherited conditions (germline, constitutional)	• Single gene and/or variant[a] • Variant confirmation[b] • Fill-in sequencing[b]	• Gene panels • Exome and/or genome	Not covered	Not covered
Cancer (somatic)			Fusion transcripts	• Liquid biopsy • Monitoring of minimal residual disease • Cancer screening
HLA typing[c]	Not covered	HLA typing	Not covered	Not covered
Prenatal testing	Not covered	Not covered	Not covered	• NIPT (aneuploidy) • Common pathogenic variants

Abbreviations: cfDNA, cell-free deoxyribonucleic acid; DNA, deoxyribonucleic acid; HLA, human leukocyte antigen; NGS, next-generation sequencing; NIPT, noninvasive prenatal testing; RNA, ribonucleic acid.
[a] Additional considerations, not covered in this guideline, might be needed for somatic mutations.
[b] The use of Sanger sequencing for somatic variant confirmation is not as common as for germline testing. However, Sanger sequencing can be used to augment NGS for regions that are difficult to sequence.
[c] Sanger sequencing can also be used for targeted HLA testing for alleles associated with adverse drug reactions (eg, HLA-B*57:01, HLA-B*15:02).

4.1 Sanger Sequencing

Although Sanger sequencing is increasingly being replaced by NGS technologies, it is still a viable method for some applications, particularly for small laboratories with limited test menus that do not require NGS platforms. These applications include:

- **Tests of limited content:** Single-gene sequencing tests can be performed when only one gene is known to cause a particular disease. In these cases, Sanger sequencing is used to detect known and novel variants, and only the technology differs from NGS. Additional guidance on developing and operating Sanger sequencing assays will be provided in a forthcoming CLSI document.
- **Fill-in sequencing:** When NGS is the primary sequencing technology, regions that are suboptimally covered might need to be interrogated by other technologies, including Sanger sequencing, if adequate coverage is critical for optimal clinical utility. If an LDT includes Sanger sequencing as a subassay, performance metrics must be based on both the primary NGS test and the Sanger sequencing subassay.