CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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MM09-A2

Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition

This document addresses diagnostic sequencing using both automated capillary-based sequencers and massively parallel sequencing instruments. Topics include specimen collection and handling; isolation and extraction of nucleic acid; template preparation; sequence generation, alignment, and assembly; validation and verification; ongoing quality assurance; and reporting results.

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

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Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition

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Abstract

Sequencing methods for genotyping have moved from the research laboratory into the clinical laboratory. Sequencing is an assay format of choice for very high-complexity genotyping, especially when hundreds or thousands of bases of genetic sequence are analyzed. Clinical and Laboratory Standards Institute document MM09-A2—*Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition* addresses diagnostic sequencing using both automated capillary electrophoresis sequencers and massively parallel sequencing instruments. Topics covered include specimen collection and handling; isolation and extraction of nucleic acid; template preparation; sequence generation, alignment, and assembly; validation and verification; ongoing QA; and reporting results.

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Foreword

Significant advances in clinical diagnostic sequencing prompted the development of the second edition of this document. The original guideline focused primarily on the establishment and use of Sanger sequencing in the clinical laboratory, which at the time was the principal means for the collection of DNA sequence data. Since publication of the first edition, massively parallel sequencing (MPS) has become part of the clinical laboratory repertoire. MPS is a catchall term that includes a number of technologies that can generate a large amount of digital sequences. A feature that distinguishes MPS from Sanger sequencing is the heavy reliance on informatics to process the raw data derived from the instrument into interpretable DNA sequence. Laboratories have already begun to establish testing using MPS for heritable conditions, cancer, and infectious diseases. Advances in other areas suggest that applications for analysis of genome-wide methylation patterns, microbiomes, metagenomics, and transcriptome sequencing are forthcoming.

This revised guideline provides additional details that address the implementation of MPS into the clinical laboratory. In Section 5, users are initially oriented to the many sequencing technologies and currently available applications; the contents of this section represent the technologies available at the time of this publication, and users are encouraged to seek out recent reviews for the latest updates in this rapidly evolving field. Information on the implementation of sequencing in the clinical laboratory has been significantly expanded. Other sections have been added that discuss issues relevant to setup, running, and QC of the instrumentation and considerations for informatics analysis. Test validation is discussed in greater detail, and a separate section on QA and QC was also created. The final section that addresses the reporting of results was revised primarily as a consequence of new guidance and resources that have become available since publication of the previous edition of MM09. Sections that address Sanger sequencing remain but have been updated as needed to reflect advances in practice. Sections addressing specimen collection and preparation for analysis were consolidated into a much shorter section (see Section 6) because these have become common laboratory practice and are covered in far greater detail in other referenced CLSI documents. This revision is designed to provide guidance to experienced and knowledgeable laboratory professionals to assist with the implementation of high-quality diagnostic sequence analysis in the clinical laboratory.

Key Words

Capillary electrophoresis, clinical sequencing, dideoxy-terminators, electrophoresis, gel electrophoresis, massively parallel sequencing, next generation sequencing, nucleic acid, polymerase chain reaction, Sanger sequencing

Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition

1 Scope

The intended users of this guideline are clinical laboratories involved in the development, validation, verification, and implementation of sequencing-based assays.

This guideline specifies recommendations for the sequencing process, including specimen collection and handling, isolation of nucleic acid, amplification and sequencing of nucleic acids, and general interpretation and reporting of genotyping results. It is the intent of this document to provide instruction for verifying that the sequence obtained is accurate and suitable for subsequent interpretation; to address general interpretation of the sequence; and to provide QA/QC considerations for each step of the process, as appropriate. It is also intended to assist laboratories in generating appropriate and efficient validation across sequencing methods and applications. Sanger-based DNA sequencing and general aspects of massively parallel sequencing (MPS) are addressed in this guideline with specific examples.

This guideline:

- Does not comprehensively address platform-specific issues, because sequencing technology is rapidly evolving
- Provides general guidance for interpreting sequencing results and does not address the medical interpretation for a given patient, which is under the purview of the health care provider
- Is relevant to germline, somatic, and microbiological applications in clinical settings

2 Introduction

Sequencing is an increasingly important tool for genotyping in molecular diagnostics. Sequencing is routinely used in genotyping infectious disease organisms such as HIV and hepatitis C virus (HCV). When typing tissue for transplantation, human leukocyte antigen (HLA) typing is also performed by sequencing. There are also a variety of applications of sequencing for oncology and for diagnosing heritable conditions. The widespread use of laboratory-developed, sequencing-based genotyping assays and commercially available sequencing-based genotyping kits spurred the development of the original guideline for the development, verification, validation, and implementation of sequencing-based assays.

The previous edition of this guideline focused on sequence analysis using dideoxy chain-terminating chemistry and capillary electrophoresis (CE) instrumentation. In recent years, a number of new technologies have been introduced commercially. One change that has occurred since the previous edition of the guideline was published is that some instruments and assays have received regulatory approval or clearance for Sanger or MPS clinical testing. For clinical tests that use these products, the process of test validation and establishing QC parameters is simplified relative to a corresponding laboratory-developed test. Additionally, there are an increasing number of clinical applications, especially for multigene panels applied to genetic disease, for specific applications in which minor population variants can be clinically important (eg, HIV tropism), and in oncology, where both broad coverage and low-level variant detection can be of value. Emerging fields, such as epigenetics and the study of the microbiomes, suggest that applications to additional complex clinical questions will only increase as sequencers are able to generate more information at lower cost. This infusion of new technology requires a fresh look at some of the subject matter covered for validation of CE sequencing applications, and also introduces new challenges in the areas of platform validation and appropriate data analysis and management. This document was