CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

MM24

Molecular Methods for Genotyping and Strain Typing of Infectious Organisms

This guideline examines the biology behind molecular strain typing and genotyping, as well as characterization and validation of these systems. It also provides recommendations regarding criteria to be considered for design, validation, and determination of clinical utility of such testing.

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

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Abstract

Molecular genotyping and strain typing are essential tools for the analysis of infectious biological agents of human diseases isolated during investigations of epidemiological outbreaks, laboratory contamination, and recurrent infection. A wide variety of genotyping and strain typing methods have been described using contemporary nucleic acid–based technologies. These methods are used for identifying virulence factors, drug resistance, markers of disease progression within an institution or a community, and in certain cases, patient prognosis. Clinical and Laboratory Standards Institute guideline MM24—*Molecular Methods for Genotyping and Strain Typing of Infectious Organisms* examines the biology behind molecular genotyping and strain typing and the process of characterizing and validating typing systems. The prevalent methods currently being used in laboratories that perform genotyping and strain typing include nucleic acid amplification tests, ribotyping, sequence-based typing (Sanger and next-generation sequencing), hybridization-based typing methods (microarrays, line-probe arrays), molecular fingerprinting (pulse-field gel electrophoresis, restriction fragment-length polymorphism analysis, multiple locus variable–number of tandem repeat analysis), and protein-based typing. Each of these areas is described in detail specific to the genotyping and strain typing of nucleic acid in clinical testing and monitoring, particularly in bacterial, fungal, and viral diseases. This guideline also includes an update on technologies used for molecular genotyping and strain typing, sample preparation, standards, calibrators, reference materials, analytical and clinical verification/validation, reporting and interpreting results, and clinical utility.

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Foreword

When CLSI documents MM10 and MM11 were published in 2006 and 2007, respectively, nucleic acid testing methods used for genotyping and strain typing, although partially overlapping, were somewhat different in scope. As such, CLSI document MM10 (which has since been withdrawn from CLSI's library) described genotyping, whereas CLSI document MM11 focused on strain typing. In the intervening years, applications introduced for performing this testing have resulted in greater similarity between genotyping in the classical sense and strain identification. These genomic approaches have allowed a growing number of medical laboratories to perform this testing without differentiating between genotyping and strain identification. This guideline reflects the technological advances made in this field and encompasses genotyping, genomic identification, and strain identification of all types of infectious disease agents.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM11-A, published in 2007. MM24 also builds on topics introduced in CLSI document MM10, published in 2006, which has since been withdrawn from CLSI's library. Several changes were made in this edition, including:

- Adding a process flow chart showing the basic steps that are followed to perform genotypic and strain typing of infectious organisms
  - Each step in the process is mapped to the relevant section in this guideline.
- Providing detailed descriptions on how genotyping and strain typing are used in different settings, in particular, genotyping and strain typing in the clinical setting vs epidemiological applications of genotyping and strain typing of infectious organisms
- Updating information on the current technologies used for genotyping and strain typing
  - Discussion of technologies that are no longer used and/or have been replaced by newer methods has been eliminated.
- Providing detailed descriptions of the quality system essentials that are relevant for laboratories that perform genotyping and/or strain typing of infectious organisms
- Adding appendixes that provide example forms used in genotyping and strain typing, additional details on various technologies, and information on relevant software and Web-based 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

5'-nuclease
Association studies
Bioinformatics
Genotyping
Linkage disequilibrium
Linkage mapping
Oligonucleotide ligation assay
Single-nucleotide polymorphism
Strain typing
Whole genome sequencing

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Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the guideline
  - Abbreviations and acronyms used in the guideline

Molecular Methods for Genotyping and Strain Typing of Infectious Organisms

1 Introduction

1.1 Scope

This guideline provides a framework for facilitating consistency in reporting genotypes and bacterial, viral, and fungal strain typing and will assist laboratories that perform these studies and the professionals applying the results. Its purpose is to present not only the technologies used but also the criteria to be considered for design, verification, validation, and determination of clinical utility of such testing. These technologies are now used in many different contexts, including:

- Clinical care settings
- Public health investigations, particularly of emerging infections
- Food and pharmaceutical industries
- Environmental analyses in the clinical setting
- Regulatory agencies

This guideline is intended for manufacturers or laboratories that develop assays, laboratories that perform assays, clinicians who use the results to diagnose or manage patients, and agencies that regulate their use. This guideline:

- Is not intended for use by research laboratories
- Is not intended to provide manufacturing guidelines
- Does not discuss the development of standard reference materials for QC use

It is recommended that this guideline be used in conjunction with CLSI documents MM03,[1] MM06,[2] MM09,[3] MM12,[4] MM13,[5] MM14,[6] MM17,[7] MM18,[8] and MM22.[9]

1.2 Background

The expanding use of molecular testing methods has enabled laboratories to characterize features of infectious agents that not only affect patient care and disease course but also allow epidemiologists to track organisms throughout the population. Genome analysis of some viruses, bacteria, and other organisms may identify variants, genes, and gene functions that have clinical effects such as patient prognosis, disease progression, and management of patient drug therapy. This information is also used epidemiologically and by regulatory agencies for surveillance, outbreak investigations, and population genetics.

Recent advances in the field of molecular diagnostic methods have enabled the expanded use of molecular genotyping and strain typing to better understand the genetics associated with pathogen gene functions and their relationship to virulence, attenuation, disease progression, and prognosis during infection, as well as drug resistance. The choice of assay format and technology platform continues to be dictated by the complexity of genetic information needed. This guideline provides recommendations for the development, verification, validation, and implementation of clinical molecular tests for genotyping or strain typing for laboratories and manufacturers. It was written during a period of prolific expansion of molecular techniques applicable to genotype and strain type testing and includes technologies in practical clinical use at the time of publication.