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MM11-A

Molecular Methods for Bacterial Strain Typing; Approved Guideline

This guideline examines the biology behind molecular strain typing and the process of characterizing and validating typing systems. The prevalent methods are described with particular attention to pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

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

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Molecular Methods for Bacterial Strain Typing; Approved Guideline

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Abstract

Molecular strain typing has become an essential tool for the analysis of bacterial pathogens obtained during investigations of epidemiologic outbreaks, laboratory contamination, and recurrent infection. A wide variety of strain typing methods have been described using contemporary DNA-based technologies. However, developing methods and generating data have proven easier than defining robust approaches for interpreting the results.

Clinical and Laboratory Standards Institute document MM11-A—*Molecular Methods for Bacterial Strain Typing; Approved Guideline* examines the biology behind molecular strain typing and the process of characterizing and validating typing systems. The prevalent methods are described with particular attention to pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Specific issues in analyzing typing data derived from these methods are discussed. The guideline offers a general approach, suitable for use in the situations commonly encountered in clinical laboratories, for interpreting and reporting molecular typing results. For selected bacterial pathogens, the application of molecular typing systems and the insights derived are considered in detail.

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Foreword

Colloquially phrased, the central question in bacterial strain typing is deceptively simple:

Are two isolates the "same" or "different"?

A more rigorous construction begins to identify the complexity:

Using a well-characterized molecular technique, are the genotypes of two isolates sufficiently similar to conclude that they represent the same strain, or sufficiently different to conclude they represent different strains?

The goal of this guideline is to provide a basis for answering this question. Among the many molecular strain typing methods that have been described, relatively few have been rigorously analyzed to define their performance characteristics. The technical and biologic reproducibility of every typing system needs to be quantitatively defined to inform the user of the reliability of clear results and the implications of the inevitable ambiguous results. Because clinically important species may differ substantially in their population structure, methods may need to be explicitly characterized for different species.

The concepts underlying molecular strain typing are derived from both evolution and epidemiology. The isolates comprising a bacterial species generally display substantial genetic diversity as a result of evolutionary divergence. Isolates that are epidemiologically related (e.g., obtained from an outbreak or during the course of infection in a single patient) are presumed to be directly and recently descended from a common ancestor and thus represent a discrete lineage or genotype.

Thus, molecular strain typing can distinguish among unrelated isolates because there is evolutionary diversity within a species, and can help identify epidemiologically related isolates because they are expected to be genetically closely related. From this perspective, there is a clear tension between the biological imperative toward divergence and variation, and the analytic need for stability and consistency. This tension defines the limits of any particular molecular strain typing procedure for resolving a given epidemiologic question. Understanding those limits is critical both to choosing a technique and interpreting the results.

Key Words

Molecular epidemiology, molecular strain typing, nucleotide sequencing, population genetics, pulsed-field gel electrophoresis

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1 Scope

Bacterial strain typing is now performed in a wide range of venues, including hospital-based clinical microbiology laboratories; federal, state, and local reference laboratories; as well as industrial and commercial laboratories. Similarly, the results of bacterial strain typing are now used in many different contexts, including clinical care settings; public health investigations, particularly of emerging infections; the food and pharmaceutical industries; and environmental analyses.

The goal of this guideline is to provide a framework that will facilitate consistency in reporting bacterial strain typing and will assist both the laboratories performing these studies and the professionals applying the results. A general approach to the analysis of molecular typing data will be presented, as well as specific criteria for interpreting typing results obtained with the most commonly used methods. This guideline will focus on techniques that analyze bacterial chromosomal DNA, particularly pulsed-field gel electrophoresis (PFGE) and nucleotide sequencing; phenotypic techniques (e.g., serotyping, phage typing) and plasmid-based methods will not be addressed.

2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol*. 1996;17(1):53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*.

3 Terminology

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all challenges to harmonization. Despite these challenges, CLSI recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

The following section provides formal definitions plus explanatory notes for key terms used in this document. During the next scheduled revision, these definitions will be reviewed again for consistency with international use, and revised as needed.

Related CLSI/NCCLS Publications*

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

MM3-A2 **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

MM9-A **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline (2004).** This document addresses automated, PCR-based, dideoxyterminator, and primer extension sequencing done on gel- or capillary-based sequencers. Topics covered include specimen collection and handling; isolation of nucleic acid; amplification and sequencing of nucleic acids; interpretation and reporting results; and quality control/assessment considerations as appropriate.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

