

Archived Document

This archived document is no longer being reviewed through the CLSI Consensus Document Development Process. However, this document is technically valid as of January 2017. Because of its value to the laboratory community, it is being retained in CLSI's library.



April 2007

MM11-A

Molecular Methods for Bacterial Strain Typing; Approved Guideline

SAMPLE

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.

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes*, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

ISBN 1-56238-634-4
ISSN 0273-3099

MM11-A
Vol. 27 No. 10
Replaces MM11-P
Vol. 26 No. 14

Molecular Methods for Bacterial Strain Typing; Approved Guideline

Volume 27 Number 10

Robert D. Arbeit, MD
Judy C. Arbique, ART(CSMLS), CLS(NCA)
Bernard Beall, PhD
Ian A. Critchley, PhD
Frederic J. Marsik, PhD
Sophie Michaud, MD, MPH, CSPQ, FRCP(C)
Christine Steward, MPH, MT(ASCP)
Fred C. Tenover, PhD, ABMM
David L. Trees, PhD

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.

Clinical and Laboratory Standards Institute (CLSI). *Molecular Methods for Bacterial Strain Typing; Approved Guideline*. CLSI document MM11-A (ISBN 1-56238-634-4). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2007.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.



Copyright ©2007 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, companion product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedure manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

Suggested Citation

CLSI. *Molecular Methods for Bacterial Strain Typing: Approved Guideline*. CLSI document MM11-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

Previous Edition:

May 2006

Reaffirmed:

April 2016

Archived:

January 2017

ISBN 1-56238-634-4
ISSN 0273-3099

Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
1 Scope.....	1
2 Standard Precautions.....	1
3 Terminology.....	1
3.1 Definitions	2
3.2 Comments on Microbiological and Epidemiologic Definitions	3
3.3 Abbreviations and Acronyms	4
4 The Biology Behind Molecular Strain Typing	5
4.1 Sources of Genetic Variation	5
4.2 Population Structure of Bacterial Species.....	5
4.3 Impact of Selective Pressure on Diversity	6
4.4 Applications of Molecular Strain Typing	6
5 Validation of Typing Methodologies.....	8
5.1 Reproducibility	8
5.2 Discriminatory Power	9
5.3 Requirements for Characterizing a Molecular Typing System.....	10
5.4 Assessment of Competency for Molecular Strain Typing	11
6 Methods for Molecular Strain Typing	11
6.1 Controls.....	11
6.2 Pulsed-Field Gel Electrophoresis (PFGE)	11
6.3 Ribotyping	20
6.4 Sequence-Based Strain Typing	21
6.5 Repetitive Sequence-Based PCR (rep-PCR)	24
7 Analyzing Electrophoretic Typing Data	26
7.1 Visual Analysis of Electrophoresis Gels.....	26
7.2 Analyzing Electrophoresis Gels by Software	26
7.3 Population Genetics and the Analysis of PFGE Patterns: a Cautionary Note	32
8 Analyzing Sequence Data	33
8.1 Percent Identity	33
8.2 Pattern Recognition.....	34
8.3 BURST	34
8.4 Sequence Analysis Methods for Evolutionary Genetics.....	34
9 Interpreting Variation in Molecular Typing.....	35
9.1 Categories of Genotypic Relatedness in Molecular Strain Typing.....	36
9.2 Step One: Identify the “Reference Isolate” or Type That Focuses the Question.....	37
9.3 Step Two: Compare Each Isolate to the Reference Isolate.....	37
9.4 Translating Genotypic Relatedness Into Epidemiologic and Clinical Relatedness	37
9.5 Comparison to the “Tenover Criteria”	38

Contents (Continued)

10 Reporting Molecular Typing Results.....38

11 General Technical Issues39

 11.1 Identifying Isolates39

 11.2 Archiving Isolates—Freezing.....40

12 Examples of Molecular Typing of Bacterial Species.....40

 12.1 *Streptococcus pyogenes*40

 12.2 *Streptococcus pneumoniae*.....45

References.....48

Summary of Delegate Comments and Committee Responses.....59

The Quality Management System Approach.....68

Related CLSI/NCCLS Publications.....69

SAMPLE

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

Molecular Methods for Bacterial Strain Typing; Approved Guideline

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.