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Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline

This guideline describes currently used analytical approaches and methodologies applied to identify the clinically important genetic characteristics responsible for disease manifestation, outcome, and response to therapy in the infectious disease setting. It also provides guidance on the 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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Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

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- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

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Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

Our standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following CLSI's established consensus procedures. Provisions in CLSI standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

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The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any document. Address comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

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Healthcare professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline

Stephen P. Day, PhD
Max Q. Arens, PhD
Timothy M. Alcorn, PhD
Andreas Bakker, PhD
Angela M. Caliendo, MD, PhD
David Hillyard, MD
Michael A. Lewinski, PhD, D(ABMM), CLSp(MB)
Marianne Van Brussel, PhD
Linda M. Weigel, PhD
Kathleen B. Whitaker, PhD

Abstract

In recent years, the use of molecular methods in microbiology has increased dramatically and now includes their use in genotyping applications that assist in identifying virulence, drug resistance, markers of disease progression, and even patient prognosis. Clinical and Laboratory Standards Institute document MM10-A—*Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline* provides guidance for the development and use of genotyping methods, such as DNA sequencing, single nucleotide polymorphism (SNP) detection, and real-time target amplification techniques of target sequences specific to particular microorganisms, and presents recommendations for quality assurance, proficiency testing, and interpretation of results.

Issues specific to the *genotyping* of nucleic acid in clinical testing and monitoring, particularly in bacterial, fungal, and viral diseases, include: an update on technologies used for molecular genotyping; preparation; standards, calibrators, and reference materials; analytical and clinical verification/validation; reporting and interpreting results; clinical utility; and recommendations for manufacturers and clinical laboratories.

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Committee Membership

Area Committee on Molecular Methods

Roberta M. Madej, MS, MT
Chairholder
Roche Molecular Systems, Inc.
Pleasanton, California

Zhimin Cao, MD, PhD
New York State Dept. of Health
Albany, New York

Maurizio Ferrari, MD
International Federation of Clinical Chemistry
Milan, Italy

Frederick S. Nolte, PhD
Emory University Hospital
Atlanta, Georgia

Timothy J. O'Leary, MD, PhD
Biomedical Laboratory Research and Development Service
Department of Veterans Affairs
Silver Spring, Maryland

Carolyn Sue Richards, PhD, FACMG
Oregon Health Sciences University
Portland, Oregon

Uwe Scherf, PhD
FDA Center for Devices and Radiological Health
Rockville, Maryland

Michael A. Zoccoli, PhD
Celera Diagnostics
Alameda, California

Advisors

Dale H. Altmiller, PhD
O.U. Medical Center
Edmond, Oklahoma

Lee Ann Baxter-Lowe, PhD
University of California, San Francisco
San Francisco, California

Mark Evans, PhD
American Medical Association
Chicago, Illinois

Cristina Gianchetti, PhD
Gen-Probe
San Diego, California

Leslie Hall, MMSc
Mayo Clinic
Rochester, Minnesota

Robert B. Jenkins, MD, PhD
Mayo Clinic
Rochester, Minnesota

Alan L. Landay, PhD
Rush University Medical Center
Chicago, Illinois

Mario Pazzagli, PhD
University of Florence
Florence, Italy

Richard S. Schifreen, PhD, DABCC
Mirus Bio Corp.
Madison, Wisconsin

Laurina O. Williams, PhD, MPH
Centers for Disease Control and Prevention
Atlanta, Georgia

Janet L. Wood, MT(ASCP)
BD Diagnostic Systems
Sparks, Maryland

Subcommittee on Genotyping for Infectious Diseases

Stephen P. Day, PhD
Chairholder
Third Wave Technologies, Inc.
Madison, Wisconsin

Max Q. Arens, PhD
Vice-Chairholder
Washington University School of Medicine
St. Louis, Missouri

Timothy M. Alcorn, PhD
BioEmerge Partners, Inc.
Raleigh, North Carolina

Andreas Bakker, PhD
Amgen Inc.
Thousand Oaks, California

Angela M. Caliendo, MD, PhD
Emory University Hospital
Atlanta, Georgia

David Hillyard, MD
ARUP
Salt Lake City, Utah

Michael A. Lewinski, PhD, D(ABMM), CLSp(MB)
Quest Diagnostics Inc.
San Juan Capistrano, California

Linda M. Weigel, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia

Kathleen B. Whitaker, PhD
FDA Center for Devices and Radiological Health
Rockville, Maryland

Staff

Clinical and Laboratory Standards Institute
Wayne, Pennsylvania

John J. Zlockie, MBA
Vice President, Standards

Lois M. Schmidt, DA
Staff Liaison

Donna M. Wilhelm
Editor

Melissa A. Lewis
Assistant Editor

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Foreword

Rapid advances in technology and techniques have resulted in a number of robust, precise, and sensitive methods for determining the presence of specific genetic changes in nucleic acid sequence. Genotyping has proven itself to be a powerful tool useful in the diagnosis, prognosis, and management of patients with infectious disease. The genotyping of HIV-1 for drug resistance is now part of the standard of care of HIV-infected patients, and the identification of methicillin resistance in *Staphylococcus aureus* has proven its utility in identifying patients being admitted into a hospital setting who require special treatment and housing requirements as part of established infection control programs. Additional uses of genotyping in the management of infectious disease continue to be applied with increasing frequency.

CLSI document MM3—*Molecular Diagnostic Methods for Infectious Diseases* and CLSI/NCCLS document MM6—*Quantitative Molecular Methods for Infectious Diseases* have established the groundwork for use of molecular diagnostic methods in microbiology and were important in driving the considerations in the development of this guideline. It is the hope of this subcommittee that this document will be used in conjunction with other relevant CLSI/NCCLS documents including: EP5—*Evaluation of Precision Performance of Quantitative Measurement Methods*; EP6—*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*; EP7—*Interference Testing in Clinical Chemistry*; EP9—*Method Comparison and Bias Estimation Using Patient Samples*; EP10—*Preliminary Evaluation of Quantitative Clinical Laboratory Methods*; EP17—*Protocols for Determination of Limits of Detection and Limits of Quantitation*; and GP10—*Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots* (see the Related CLSI/NCCLS Publications section in the back of this document). While these are excellent resources, a guideline is needed to provide information specific for genotyping in infectious diseases.

A Note on Terminology

Clinical and Laboratory Standards Institute (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 obstacles to harmonization. Despite these obstacles, 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.

In keeping with CLSI's commitment to align terminology with that of ISO, the following terms are used in MM10: *trueness* is used in this document when referring to the closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand; the measurement of trueness is usually expressed in terms of *bias*; *repeatability* has replaced the term *within-run precision* where appropriate, when describing the closeness of agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement; *measuring range* has replaced *reportable range* when referring to a set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits. The ISO terms *diagnostic sensitivity* and *diagnostic specificity* have replaced *clinical sensitivity* and *clinical specificity*. In Europe, for the most part, the term *clinical* is applied to the evaluation of medical products used on or in patients, or when referring to clinical studies of drugs, under much more stringent conditions.

Users of MM10-A should understand, however, that the fundamental meanings of the terms are similar and to facilitate understanding, terms are defined along with explanatory notes in the guideline's Definitions section.

Key Words

Amplification, genotyping, mutation, nucleic acid, sequencing, signal, SNP, target

Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline

1 Scope

This guideline provides currently used analytical approaches and methodologies applied to identify the clinically important genetic characteristics responsible for disease manifestation, outcome, and response to therapy in the infectious disease setting. Its purpose is to present not only the technologies utilized, but also the criteria to be considered for design, verification, validation, and determination of clinical utility of such testing. It is recommended that this guideline be used in conjunction with the following CLSI/NCCLS documents: MM3—*Molecular Diagnostic Methods for Infectious Diseases*; MM6—*Quantitative Molecular Methods for Infectious Diseases*; MM9—*Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine*; and MM13—*Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods.*

This document 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.

2 Introduction

The expanding utilization of molecular testing methods has afforded laboratories the means to characterize clinically important features of infectious agents that impact patient care and disease course. Analysis of the genomes of certain viruses, bacteria, and other organisms may identify mutations, genes, and gene functions that can ultimately affect patient prognosis, disease progression, and management of patient drug therapy.

Recent advances in the field of molecular diagnostic methodologies have allowed the expanded use of these techniques to better understand the genetics associated with microbial gene functions, their relationship to virulence, attenuation, disease progression, and prognosis during infection, as well as antimicrobial drug resistance. This genotypic testing has demonstrated clinical utility in patient management and has resulted in genotyping moving rapidly out of the research laboratory and into the clinical laboratory setting. The choice of assay format and technology platform continues to be dictated by the complexity of genetic information required to obtain a result. With the increase in the use of laboratory-developed ("home-brew") genotyping assays and commercially available kit-based genotyping assays, a guideline for the development, verification, validation, and implementation of clinical genotypic tests is required to guide laboratories and manufacturers. It is hoped that this guideline will serve as a roadmap for laboratories to use in guiding themselves in implementing genotypic testing. This document was written during a period of prolific expansion of molecular techniques applicable to genotypic testing and it addresses only those technologies in practical clinical use at the time the document was drafted.

3 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

