MM18-A
Vol. 28 No. 12
Replaces MM18-P
Vol. 27 No. 22

Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline

Sequencing DNA targets of cultured isolates provides a quantitative metric within which to perceive microbial diversity, and can serve as the basis to identify microorganisms. This document is an effort to catalyze the entry of molecular microbiology into clinical usage by establishing interpretive criteria for microorganism identification.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®
(Formerly NCCLS)

MM18-A
ISBN 1-56238-664-6
Volume 28 Number 12 ISSN 0273-3099

Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline

Cathy A. Petti, MD
Philipp P. Bosshard, PhD
Mary E. Brandt, PhD
Jill E. Clarridge, III, PhD
Tamara V. Feldblyum, MS
Paul Foxall, PhD
Manohar R. Furtado, PhD
Norman Pace, PhD
Gary Procop, MD

Abstract

The information presented in this document is intended for use with molecular diagnostic testing procedures published in CLSI guideline MM3 and CLSI/NCCLS guideline MM9. The guidelines contain information about the development, evaluation, and application of nucleic acid-based testing for infectious diseases and chemistries for diagnostic laboratories.

Laboratories often receive clinical isolates for bacterial and fungal identification that have ambiguous biochemical profiles by conventional testing. The identification of microorganisms historically has relied on phenotypic methods. Because of the growing microbial diversity with emergence of common pathogens having rare or unique phenotypic characteristics and new pathogenic microorganisms with poorly defined phenotypes, conventional methods often cannot fully characterize bacterial or fungal isolates, and laboratories are now relying on broad-range DNA sequencing for microorganism identification. The information here represents the most current information for microbial classification by DNA target sequencing, with particular emphasis on interpretation and reporting results.

Clinical and Laboratory Standards Institute (CLSI). *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline*. CLSI document MM18-A (ISBN 1-56238-664-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.

Contents

Foreword

Many laboratories now use sequencing for the identification of bacteria (aerobic bacteria, anaerobic bacteria, and mycobacteria) and fungi, but the implementation of broad-range DNA sequencing for routine clinical use has not been well delineated. Two related documents, CLSI/NCCLS document MM9 and CLSI document MM10,[1,2] are important contributions to this field, but their sections on reporting and interpreting results do not adequately address identification of microorganisms by broad-range DNA (eg, 16S rRNA, fungal internal transcribed spacer [ITS] regions) sequencing. Understandably, taxonomy based on this method is an evolving field, but a need exists to develop a systematic and uniform approach to identifying microorganisms by broad-range DNA sequencing for clinical laboratories. Although the taxonomical classifications are not always clear, a consensus document on DNA target sequencing will unify the approach for purposes of consistent and standardized reporting across all clinical laboratories.

In this document, guidelines are established for implementing target sequencing, with an emphasis on 16S rRNA gene for bacteria and ITS regions for fungi. This guideline reviews (1) selection of DNA target sequence; (2) sequence length; (3) quality of generated sequence (ambiguous bases and intracellular polymorphisms); (4) intergenus, intragenus, interspecies, and intraspecies variability of microorganisms; and (5) selection of reference databases. Additionally, the impact of these variables on microorganism identification is discussed, with emphasis on microorganisms that are clinically relevant or commonly encountered in a clinical laboratory.

Interpretive criteria for defining genus and species have not been consistent in the literature, and often vary with the queried microorganism. Since defining absolute interpretive criteria can be complex and highly nuanced, this document establishes guidelines for the systematic *approach* to classify bacteria and fungi by broad-range DNA sequencing.

Key Words

16S rRNA, bacterial identification, broad-range primer, fungal identification, gene sequencing, ITS, nucleic acid amplification

Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline

1 Scope

This guideline specifies recommendations for clinical laboratories that employ amplification and Sanger-based (dideoxy-termination) sequencing of broad-range DNA targets for the identification of bacteria, mycobacteria, and fungi from cultured clinical isolates. Partial and full gene sequencing with 16S rRNA gene for identification of bacteria and mycobacteria, and internal transcribed spacer regions ITS1 and ITS2 regions for identification of fungi, are addressed with inclusion of alternative DNA targets when appropriate. To assist the clinical laboratory, this document provides guidelines for:

- selection of DNA targets and size of targets for amplification and sequencing;
- establishment of quality control parameters for amplification and sequencing;
- measurement of quality of sequence;
- assessment of reference sequences and databases;
- comparison of sequences for identification;
- establishment of interpretive criteria for identity scores generated by gene sequencing;
- reporting strategies that are clinically relevant for specific groups of microorganisms; and
- limitations of gene sequencing for microbial identification.

This guideline is not intended to:

- address RNA targets for sequencing;
- provide guidelines for definitive taxonomical criteria for classification of microorganisms or methods to identify novel microorganisms;
- address alternative sequencing systems or specific molecular assays designed with these broad-range DNA targets;
- type strains for epidemiological purposes;
- identify viruses or parasites; or
- address amplification and sequencing from direct specimens.

2 Introduction

Microbial taxonomy has undergone a revolution over the past few decades as a consequence of the availability of gene and even genome sequences. Comparison of gene sequences from different organisms provides a quantitative metric within which to perceive microbial diversity and to classify diverse organisms. Gene sequences also serve as the basis of molecular tools for sensitive and incisive

identification of organisms. Highly conserved genes, such as the ribosomal RNA (rRNA) genes, provide information on the general properties of organisms based on the properties of their known relatives. Other gene sequences can provide further detail, eg, as may be needed to distinguish a pathogen from a harmless commensal microbe or to identify antibiotic resistant strains of bacteria.

In contrast to the precision of sequence comparisons, microorganisms traditionally have been identified by their physiological properties as perceived in pure cultures. Phenotypic properties often are anecdotal, however, and slowly growing or difficult-to-culture microbes pose additional barriers and expenses to clinical identification. Thus, the emerging molecular technology offers much that can complement the practice of the clinical microbiologist. Gene sequence-based techniques can serve to detect, identify, and monitor microbes, even poorly known or uncultured pathogens.

Molecular technology has made some inroads into clinical microbiology, through tests for specific pathogens such as *Mycobacterium tuberculosis*. Nonetheless, few applications of sequence analyses have come into general clinical usage. Although molecular methods promise a new dimension to the clinical microbiology laboratory, the technology is not widely distributed and the significance of molecular identification results can be confusing to clinicians. Critically, there are no standardized methods for conducting analyses and interpreting the results. This document is an effort to catalyze the entry of molecular microbiology into clinical usage. Developments in this arena are only beginning, but already the prospects are bright for more rapid, accurate, and economical identification of clinically relevant microorganisms than currently is achieved.

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 US Centers for Disease Control and Prevention.[3] 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 CLSI document M29.[4]

All microorganisms should be handled at the appropriate biosafety level practicing standard and universal precautions guidelines (*Biosafety in Microbiological and Biomedical Laboratories*, US Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. 5th ed. 2007. http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl5toc.htm).[3]

Standard precautions for molecular diagnostic testing of infectious disease agents must be exercised according to CLSI document MM3.[5]

4 Terminology

4.1 Definitions

alignment – the process of lining up two or more sequences for the purpose of assessing the percent identity shared between sequences.

amplicon//amplification product – the relatively low molecular weight product created from a target amplification reaction; **NOTE:** PCR (polymerase chain reactions) produce double-stranded DNA

Related CLSI Reference Materials*

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, dideoxy-terminator, 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 of results; and quality control/assessment considerations as appropriate.

MM10-A **Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline (2006).** 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.

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

