CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

MM18

Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing

This guideline includes information on sequencing DNA targets of cultured isolates, provides a quantitative metric for perceiving microbial diversity, and can serve as the basis to identify microorganisms. By establishing interpretive criteria for microorganism identification by targeted DNA sequencing, this guideline provides structure to laboratories that identify microorganisms for medical use.

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

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MM18, 2nd ed.
July 2018
Replaces MM18-A

Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing

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Abstract

Clinical and Laboratory Standards Institute guideline MM18—*Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing* includes information intended for use with molecular diagnostic testing procedures published in CLSI documents MM03[1] and MM09.[2] These guidelines contain information about developing, evaluating, and applying nucleic acid–based testing for infectious diseases in medical laboratories.

Historically, microorganism identification has relied on phenotypic methods and, more recently, matrix-assisted laser desorption/ionization time-of-flight technology. Patient isolates for bacterial and fungal identification may have ambiguous biochemical profiles or mass spectra and cannot be reliably characterized. Laboratories can apply broad-range DNA sequencing for microorganism identification and as a standardized, portable method for data sharing. This guideline includes the most current information for microbial classification by targeted DNA sequencing, with particular emphasis on interpreting and reporting results.

Clinical and Laboratory Standards Institute (CLSI). *Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing*. 2nd ed. CLSI guideline MM18 (ISBN 978-1-68440-012-6 [Print]; ISBN 978-1-68440-013-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing.* 2nd ed. CLSI guideline MM18. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Previous Editions:
August 2007, April 2008

ISBN 978-1-68440-012-6 (Print)
ISBN 978-1-68440-013-3 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 38, Number 15

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Acknowledgment for the Expert Panel on Molecular Methods

CLSI, the Consensus Council, and the Document Development Committee on Interpretive Criteria gratefully acknowledge the Expert Panel on Molecular Methods for serving as technical advisors and subject matter experts during the development of this guideline.

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Foreword

Many laboratories use targeted DNA sequencing for bacterial (aerobic, anaerobic, and mycobacteria) and fungal identification, particularly for isolates that are poorly characterized by growth-dependent or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) methods. Although CLSI document MM09[2] provides important contributions to this field, MM18 specifically focuses on reporting and interpreting results for identifying microorganisms by broad-range targeted DNA (eg, 16S ribosomal RNA, fungal internal transcribed spacer regions) sequencing. As this taxonomy-based method continues to evolve, this guideline provides recommendations for practically applying sequence-based technologies in the medical laboratory. Although the taxonomical classifications are not always clear, a consensus guideline on targeted DNA sequencing provides a systematic and uniform approach for consistently reporting standardized results across all medical laboratories.

Interpretive criteria for defining genus and species have been inconsistent in the literature, often varying with the queried microorganism. Because defining absolute interpretive criteria can be complex and highly nuanced, this guideline establishes recommendations for the systematic approach to classifying bacteria and fungi by broad-range DNA sequencing.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM18-A, published in 2008. Several changes were made in this edition, including:

- Reorganized to fit the CLSI quality management system and path of workflow format
- Revised all bacteriology tables (Tables 6 to 16) to reflect current taxonomy and to outline where sequencing of the 16S rRNA gene's V1-V3 region (ie, first ≈ 500 base pairs) provides genus- and/or species-level identification and where diversity occurs within the entire gene to distinguish each genus and/or species
- Deleted table on bacterial agents of bioterrorism and its introductory text and added discussion of each agent to the group-specific tables (Tables 6 to 16)
- Revised all organism tables to include information on how MALDI-TOF MS may be used to complement sequencing for identification
- Deleted all dendrograms
- Revised organism tables to include emerging, clinically relevant microorganisms
- Updated organism nomenclature

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

Bacterial identification, fungal identification, gene sequencing, internal transcribed spacer, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, nucleic acid amplification, primer, 16S rRNA

Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline specifies recommendations for interpreting and reporting results of Sanger-based (dideoxynucleotide chain termination) sequencing of broad-range DNA targets for identifying pure isolates of bacteria, mycobacteria, and fungi from cultured patient isolates. Partial- and full-gene sequencing with 16S ribosomal RNA (rRNA) genes for bacterial and mycobacterial identification, as well as internal transcribed spacer (ITS) regions (ie, ITS-1 and ITS-2) for fungal identification are covered, including alternative DNA targets when appropriate. Although massively parallel (next-generation) sequencing technologies are rapidly emerging, this guideline's scope is limited to Sanger-based targeted DNA sequencing.

To assist the medical laboratory, guidance is provided for:

- Selecting DNA targets and sizes for amplification and sequencing
- Establishing QC parameters for amplification and sequencing
- Measuring sequence quality
- Assessing reference sequences and databases
- Comparing sequences for identification
- Establishing interpretive criteria for identity scores generated by targeted DNA sequencing
- Developing clinically relevant reporting strategies for specific microorganism groups
- Identifying the limitations of targeted DNA sequencing for microbial identification

The intended users of this guideline are medical laboratories and laboratories performing amplification and Sanger-based (dideoxynucleotide chain termination) sequencing of broad-range DNA targets for identifying bacteria, mycobacteria, and fungi from cultured patient isolates.

This guideline does not:

- Include procedures for performing microbial sequencing.
- Include RNA targets for sequencing.