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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

Advancing Quality in Health Care Testing

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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.

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Contents

Abstract..... i

Committee Membership..... iii

Foreword..... vii

1 Scope..... 1

2 Introduction..... 1

3 Standard Precautions..... 2

4 Terminology..... 2

 4.1 Definitions 2

 4.2 Abbreviations and Acronyms 5

5 Approach to DNA Target Sequencing..... 5

 5.1 Primer Design 5

 5.2 Controls..... 7

 5.3 Protocol and Instrument Requirements..... 8

 5.4 Overview of Sequence Data..... 9

 5.5 Selection of Reference Databases 11

 5.6 Sequence Comparison for Identification 12

 5.7 Generalized Approach to Sequence-Based Identification for Bacteria (excluding Mycobacteria and Actinomycetes) 14

6 Interpretive Criteria of Identity Scores 16

 6.1 Staphylococci and Related Gram-Positive Cocci 16

 6.2 *Streptococcus* spp., *Enterococcus* spp., and Streptococcal-Like Organisms..... 21

 6.3 Glucose Nonfermenting Gram-Negative Bacilli..... 30

 6.4 Campylobacterales..... 35

 6.5 Gram-Positive Anaerobes 37

 6.6 Gram-Negative Anaerobes..... 40

 6.7 Coryneform Gram-Positive Bacilli 43

 6.8 Aerobic Actinomycetes..... 45

 6.9 *Mycobacterium* sp..... 48

 6.10 Bacterial Agents of Bioterrorism 52

 6.11 Fungi..... 54

7 Suggestions for Result Reporting 59

References..... 60

Summary of Delegate Comments and Committee Responses 68

The Quality Management System Approach 70

Related CLSI Reference Materials 71

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.

The findings and conclusions in this Clinical and Laboratory Standards Institute (CLSI) guideline are those of the subcommittee contributing authors and participants in the consensus process, and do not necessarily represent the views of the Centers for Disease Control and Prevention (CDC).

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