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MM18-P
Vol. 27 No. 22

Interpretive Criteria for Microorganism Identification by DNA Target Sequencing; Proposed Guideline

PLEASE

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This proposed document is published for wide and thorough review in the new, accelerated Clinical and Laboratory Standards Institute (CLSI) consensus-review process. The document will undergo concurrent consensus review, Board review, and delegate voting (i.e., candidate for advancement) for 60 days.

Please send your comments on scope, approach, and technical and editorial content to CLSI.

Comment period ends

23 October 2007

The subcommittee responsible for this document will assess all comments received by the end of the comment period. Based on this assessment, a new version of the document will be issued. Readers are encouraged to send their comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; Fax: +610.688.0700, or to the following e-mail address: customerservice@clsi.org

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COMMENT

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.

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Advancing Quality in Healthcare Testing

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In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

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.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The CLSI voluntary consensus process is a protocol establishing formal criteria for:

- 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.

Proposed A consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

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.

COMMENTS

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.

VOLUNTEER PARTICIPATION

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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Interpretive Criteria for Microorganism Identification by DNA Target Sequencing; Proposed Guideline

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Abstract

The information presented in this document is intended for use with molecular diagnostic testing procedures published in the following CLSI approved guidelines: MM3-A2—*Molecular Diagnostic Methods for Infectious Diseases: Approved Guideline—Second Edition*; MM9-A—*Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine: Approved Guideline*. 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 gene sequencing, with particular emphasis on interpretation and reporting results.

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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 healthcare 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/NCCLS 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

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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 CLSI documents[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 (e.g., 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 (4) 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 interpretative 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.

Invitation for Participation in the Consensus Process

An important aspect of the development of this and all CLSI documents should be emphasized, and that is the consensus process. Within the context and operation of CLSI, the term “consensus” means more than agreement. In the context of document development, “consensus” is a process by which CLSI, its members, and interested parties (1) have the opportunity to review and to comment on any CLSI publication; and (2) are assured that their comments will be given serious, competent consideration. Any CLSI document will evolve as will technology affecting laboratory or healthcare procedures, methods, and protocols; and therefore, is expected to undergo cycles of evaluation and modification.

The Area Committee on Molecular Methods has attempted to engage the broadest possible worldwide representation in committee deliberations. Consequently, it is reasonable to expect that issues remain unresolved at the time of publication at the proposed level. The review and comment process is the mechanism for resolving such issues.

The CLSI voluntary consensus process is dependent upon the expertise of worldwide reviewers whose comments add value to the effort. At the end of a 60-day comment period, each subcommittee is obligated to review all comments and to respond in writing to all which are substantive. Where appropriate, modifications will be made to the document, and all comments along with the subcommittee’s responses will be included as an appendix to the document when it is published at the next consensus level.

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).

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Key Words

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

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Interpretive Criteria for Microorganism Identification by DNA Target Sequencing; Proposed 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

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