MM17-P
Vol. 27 No. 21

Verification and Validation of Multiplex Nucleic Acid Assays; 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

28 September 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

This guideline provides recommendations for analytic verification and validation of multiplex assays, as well as a review of different types of biologic and synthetic reference materials.

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

(Formerly NCCLS)

Clinical and Laboratory Standards Institute

Advancing Quality in Healthcare Testing

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

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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Abstract

Multiplex assays detect the presence of and discriminate two or more analytes simultaneously in the same sample. The number of commercially available multiplex assays is increasing rapidly, as is the number of laboratory-developed multiplex assays, and these use a variety of technologies and instrument platforms. Multiplex testing provides significant challenges to the laboratory with regards to appropriate verification and validation testing, and especially the acquisition of appropriate control and reference materials to conduct the testing. The complexity of data analysis and reporting of results is increased relative to single-result assays. CLSI document MM17-P—*Verification and Validation of Multiplex Nucleic Acid Assays; Proposed Guideline* addresses analytical verification and validation of qualitative and semiquantitative multiplex nucleic acid assays; however, verification and validation of quantitative assays or expression assays are not addressed in this guideline. Topics covered include sample preparation, a general discussion of multiplex methods and technologies, reference and quality control materials, analytic verification and validation, data analysis, and reporting of results. Clinical validation is briefly reviewed. Specific protocols for verification and validation are not given, due to the variety and breadth of multiplex testing; but detailed recommendations, based on the most current guidance documents, for appropriate analytical verification and validation are provided.

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Foreword

Nucleic acid testing is the fastest growing field in laboratory medicine. The first generation of nucleic acid tests concentrated on measuring the presence or the quantification of a single target, often using a single internal control. More recently, the field of multiplex nucleic acid testing has expanded greatly for both laboratory-developed and commercially marketed tests. Applications of multiplex technology occur in many different clinical areas: carrier screening for genetic diseases (e.g., cystic fibrosis mutations, Ashkenazi Jewish mutation panels and thrombosis panels); pharmacogenomics (e.g., cytochrome P450 genotyping and single nucleotide polymorphisms that discriminate different rates of warfarin metabolism); infectious disease panels (e.g., for respiratory pathogens and gastrointestinal tract infections); pathogen genotyping (e.g., hepatitis C virus [HCV]); and genetic risk applications (e.g., breast cancer prognosis and therapy). These assays use a variety of platforms and technologies, and measure both DNA and RNA targets. While the chemistry of the different technologies applied to multiplex nucleic assays may be different, sample handling, control strategy, data assessment, and reporting of the results are independent of any set of reagents that might be used. For this guideline, multiplex assays are defined as assays where at least two targets are simultaneously detected through a common process of sample preparation, amplification (target or signal), detection, and interpretation. This guideline is limited to a discussion of multiplex assays for genotyping and pathogen detection, and excludes extensive discussion of gene expression assays.

For a multiplex nucleic acid test to reliably achieve its intended use, there must be control of the process from the acquisition of the sample and preparation of the nucleic acid for testing to the evaluation of the data and the reporting of the results. The competition among reactions in multiplex assays may impose more stringent requirements for sample purity, sample input, reagents, and platforms to avoid nonspecific reactions and background signal. In comparison to single analyte assays, multiplex assays require an increased number of controls, more complex performance evaluation/data analysis algorithms, and more complex reporting of results. The availability of sufficient and appropriate control and reference materials to properly verify and validate multiplex nucleic acid tests is a major challenge. This guideline provides recommendations for various aspects of verification and validation of multiplex testing, and also includes a general overview of technologies currently in use for multiplex testing. The types of control and reference materials that may be available for verification, validation, and daily quality control testing for multiplex assays are extensively discussed. Adequate performance evaluation, as well as interpretation and reporting of multiplex testing results, is still evolving, and additional guidance documents from regulatory agencies and guidelines from standards organizations will surely appear; but this guideline provides the best recommendations available at this time. Overall, this guideline is designed to assist laboratories and manufacturers in developing, verifying, validating, and implementing multiplex nucleic acid tests for diagnostic use.

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.

A Note on Terminology

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 challenges to harmonization. In light of this, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

Key Words

Genotyping, laboratory-developed assay, multiplex, multiplex assay, validation, verification

Verification and Validation of Multiplex Nucleic Acid Assays; Proposed Guideline

1 Scope

Multiplex assays generate multiple, simultaneous results on a single sample such that two or more mutations are simultaneously examined in either a single or many genes; examples include cystic fibrosis transmembrane conductance regulator *(CFTR)* mutation analysis, a panel of several loci with common mutations in the Ashkenazi Jewish population, cytochrome P-450 genotyping, deep vein thrombosis panels, and hepatitis C virus (HCV) genotyping. Multiplex testing provides significant challenges to the laboratory with regards to appropriate verification and validation. A variety of technologies and instrument platforms are available for performing multiplex assays. It is important to note that acquisition of appropriate biological control materials can be extremely difficult or impossible. Moreover, the complexity of data analysis and reporting of results is increased relative to singleplex assays.

This guideline provides recommendations for analytic verification and validation of qualitative multiplex assays, as well as a review of different types of biologic and synthetic reference materials (RM). Multiplex assays are defined as those in which two or more targets are simultaneously detected through a common process of sample preparation, target or signal amplification, allele discrimination, and collective interpretation. An overview of currently available technologies, as well as recommendations for evaluating new ones, is provided. An extensive review of the design, acquisition, and appropriate use of different types of control materials, including blood samples, residual patient samples, products of whole genome amplification, synthetic oligonucleotide simplex and multiplex controls, and plasmids, is provided. Current assay formats are used to illustrate proper verification and validation protocols, and appropriate data analysis and result reporting for multiplex assays are described.

This guideline does not address assays that measure individual targets that are then evaluated simultaneously, and is limited to a discussion of analytic validation and verification of qualitative multiplex assays for genotyping and pathogen detection/identification. Extensive discussion of validation and verification of gene expression assays, which may be a subject of a separate guideline, is excluded; however, many aspects of this guideline may also apply to expression assays, such as specimen handling, sample preparation, nucleic acid isolation, amplification and detection technologies, and instrument platforms. This guideline focuses on analytical performance and only includes a general discussion on clinical performance. A general overview of multiplex technologies is provided to the extent of discussing unique aspects and special considerations for multiplex assays, but does not discuss in detail the basic technologies, which are covered in other Molecular Methods guidelines (i.e., this guideline does not specifically address many microarray-based detection platforms, which are the subject of a separate CLSI document).[1] See the related CLSI documents on this subject.[1-3]

2 Introduction

With the complete sequencing of the human genome and ever increasing numbers of viral and bacterial genomes, as well as the development of the associated fields of genomics and pharmacogenomics, there has been a rapid expansion of genotyping assays available in the clinical laboratories. More importantly, genotyping assays are increasingly run as multiplex assays (i.e., the simultaneous detection of two or more targets on the same sample in a single reaction). Multiplex assays are used for detection and identification of infectious agents (pathogen panels), identification of genetic disorders (targeted mutation panels), choosing drug therapies and doses (pharmacogenetic panels), and assessing disease progression and prognosis (genetic association panels). Irrespective of the clinical use, all multiplex assays present the clinical laboratory with significant challenges in verification and validation, acquisition of appropriate control materials, data analysis, and reporting. Laboratories can develop assays in-house ("home-brew,

