CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

MM17

Validation and Verification of Multiplex Nucleic Acid Assays

This guideline includes recommendations for analytical validation and verification of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

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

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Validation and Verification of Multiplex Nucleic Acid Assays

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Abstract

Clinical and Laboratory Standards Institute guideline MM17—*Validation and Verification of Multiplex Nucleic Acid Assays* discusses analytical validation and verification of qualitative multiplex nucleic acid assays. Topics covered include sample preparation, a general discussion of multiplex methods and technologies, reference and quality control materials, data analysis, and results reporting. Clinical validity and utility are briefly reviewed. Because of the variety and breadth of multiplex testing, specific protocols for validation and verification are not included. However, detailed recommendations for appropriate analytical validation and verification, based on the most current guidance documents, are provided.

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Contents

Foreword

Nucleic acid testing is one of the fastest growing fields in laboratory medicine. First-generation nucleic acid tests concentrated on measuring the presence or quantity of a single target, often using a single internal control. Recently, the multiplex nucleic acid testing field has expanded greatly for both laboratory-developed and marketed tests.

These assays use various platforms and technologies and measure both DNA and RNA targets. Although the chemistry technologies applied to multiplex nucleic assays may be different, sample handling, control strategies, data assessment, and results reporting are independent of any reagent set that might be used. In this guideline, multiplex assays are defined as assays in which two or more targets are simultaneously detected through a common process of sample preparation, amplification (target or signal), detection, and interpretation.

For a multiplex nucleic acid test to reliably achieve its intended use, process control is needed from sample acquisition and nucleic acid preparation for testing to data evaluation and results reporting. The competition among reactions in multiplex assays may necessitate more stringent requirements for sample purity, sample input, reagents, and platforms to avoid nonspecific reactions and background signal. Compared with single measurand assays, multiplex assays need more controls, more complex performance evaluation and data analysis algorithms, and more complex results reporting. Obtaining sufficient and appropriate control and reference materials (RMs) to properly validate and verify multiplex nucleic acid tests is a major challenge.

This guideline is designed to assist laboratories and manufacturers in developing, validating, verifying, controlling, analyzing, and implementing multiplex nucleic acid tests for diagnostic use. It provides recommendations for various aspects of multiplex test validation and verification and also includes a general overview of technologies currently in use for multiplex testing. The types of control and RMs that may be available for validation, verification, and daily quality control testing for multiplex assays are extensively discussed. Evaluation of adequate performance, as well as interpretation and reporting of multiplex testing results, is still evolving, and additional guidance documents from regulatory and standards organizations need to be developed. However, this guideline provides the most up-to-date recommendations currently available.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM17-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
- Moved technologies overview to Appendix A
- Provided detailed, updated information on specimen types
- Added or revised information on RM types and uses
- Included guidance on using an error-based approach to validation and verification

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

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

Validation and Verification of Multiplex Nucleic Acid Assays

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- "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 provides recommendations for qualitative multiplex nucleic acid assay validation and verification. This guideline focuses primarily on analytical validation, analytical verification, and QC and briefly discusses establishing clinical validation and clinical verification for these assays. The intended audience includes laboratory directors, medical microbiologists, laboratory technologists, QA personnel, and assay manufacturers. This guideline is not intended to be regulatory guidance but to provide current best practice recommendations. Additional regulatory and/or accreditation requirements may apply.

The design, acquisition, and appropriate use of different control materials are extensively reviewed. Current assay formats are used to illustrate proper validation and verification protocols, and appropriate data analysis and results reporting for multiplex assays are described. Because traditional single-measurand protocols are difficult or impossible to perform with multiplex assays, an error-based approach to validation and verification is presented. This error-based approach may be applicable to multiplex assays performed with a single test method, for which the performance characteristics for different measurands are expected to be similar.

This guideline describes general considerations and recommendations for multiplex testing platforms but does not discuss some basic technologies covered in detail in other CLSI molecular methods guidelines (eg, this guideline does not specifically discuss many microarray-based detection platforms or next-generation sequencing). Appendix A provides an overview of some currently available multiplex testing technologies. For additional information, see CLSI documents MM01,[1] MM03,[2] MM06,[3] MM09,[4] MM21,[5] MM22,[6] and MM23.[7]

This guideline discusses multiplex assays for genotyping and pathogen detection and excludes gene expression assays. This guideline also does not cover assays measuring individual targets that are then evaluated together to yield a composite score or classifier as a result.