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Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition

This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse-transcriptase PCR techniques, and includes information on specimen collection, sample preparation, test reporting, test validation, and quality assurance.

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

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Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition

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Abstract

Analysis of nucleic acids is playing an increasing role in the diagnosis and management of patients with hematopoietic neoplasms. The tests include those for detection of clonality by analysis of gene rearrangements in the antigen receptor genes or detection of nonrandom inactivation of the X chromosome, detection and quantification of junctions formed by chromosomal translocations, detection of micromutations, quantification of chimerism after allogeneic hematopoietic transplantation, and quantification of normal DNA or RNA sequences. The methods used in clinical molecular hematopathology include end-point PCR, reverse-transcriptase PCR, real-time fluorescence-based PCR, DNA sequencing, FISH, and hybridization-based microarray and microbead assays.

Clinical and Laboratory Standards Institute document MM05-A2—*Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition* addresses the needs of the laboratory by providing recommendations on a variety of laboratory tests based on analysis of nucleic acids. It addresses preexamination and examination issues affecting assay performance, reporting of laboratory results, and QA. The guideline is designed to assist a molecular diagnostic laboratory in acquiring a new assay or new technology, as well as serve as a refresher for those already experienced with a particular area of analysis.

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Foreword

The Document Development Committee on Nucleic Acid Amplification Assays for Molecular Hematopathology was formed to address the need for a guideline on the performance and interpretation of molecular assays in diagnostic hematopathology. Since the previous edition of this guideline, both technology and the understanding of the molecular basis of hematological malignancies have evolved considerably. In this guideline, the current state of molecular diagnostic techniques used for the diagnosis of hematological disorders, including clonality analysis, detection of mutations and chromosomal rearrangements associated with specific neoplasms, and assays used for prognostication and clinical monitoring of patients with hematological neoplasms, are detailed. This guideline is not intended to include molecular diagnostic techniques for all hematological disorders, particularly inherited genetic hematological diseases. In addition, due to the rapidly changing nature of molecular diagnostics, omissions in this document due to the development of new techniques after publication are possible.

The methods and QC approaches described herein are not absolute or immutable. They represent recommendations presented by the document development committee, and are intended for use by diagnostic laboratories. Such use is intended to facilitate both interlaboratory comparisons of results and diagnostic interpretations, as well as to ensure accuracy in diagnosis.

Key Words

Allogeneic stem cell transplant, amplification, clonality, DNA sequencing, fusion gene, gene rearrangement, hematopathology, immunoglobulin, leukemia, lymphoma, mutation detection, nucleic acid, polymerase chain reaction, real-time polymerase chain reaction, reverse transcription, Southern blot, T-cell receptor translocation

Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition

1 Scope

The use of molecular methods in clinical diagnosis has become an essential part of the practice of pathology. In hematopathology, molecular methods are used to identify clonal cell proliferations, chromosomal translocations, the production of abnormal RNA species, somatic mutations of neoplastic cell populations, and quantification of abnormal nucleic acids. Along with the development of new testing methods, molecular pathologists have actively worked to determine the clinical scenarios in which molecular diagnostic testing is indicated and to improve the quality of molecular diagnostic tests. To assure the continued success of nucleic acid–based diagnostics, several key areas warrant attention.

This guideline is written for laboratory directors, surgical pathologists, medical technologists, other laboratory personnel, hematopathologists, hematologists, oncologists, and those involved in the promulgation of regulations under which laboratories and manufacturers must operate.

This guideline is intended to assist laboratories that rely on nucleic acid–based hematology assay systems to properly implement these techniques, together with the appropriate controls in their laboratories. Furthermore, it is intended to help the laboratorian determine what types of materials and records must be preserved following the laboratory procedure, and for how long. Finally, it is intended to assist those responsible for monitoring compliance with QA programs.

This document addresses the following topics as they relate to molecular detection of lymphoid and myeloid clonality, chromosomal translocations, somatic mutations in lymphoid and myeloid neoplasms, and quantification of donor/recipient cell populations after allogeneic transplants, mutations/translocations, and normal RNA species:

- Indications for molecular diagnostic testing
- Specimen collection, transport, and processing
- Assessment of specimen adequacy
- Conduct of molecular hematology assays, and sensitivity, specificity, controls, and artifacts
- QA
- Interpretation of results

This document does not address genetic testing for inherited mutations. Refer to CLSI document MM01[1] for genetic testing guidelines. This document also does not address procedures for validation of molecular diagnostic tests. For information on validation of molecular diagnostic tests, refer to CLSI documents C28,[2] EP05,[3] EP06,[4] EP09,[5] EP12,[6] EP15,[7] EP17,[8] MM01,[1] MM03,[9] and MM06.[10] In addition, this document does not discuss next generation sequencing; therefore, discussion of sequencing or direct sequencing is in reference to Sanger sequencing.

2 Introduction

2.1 Diagnostic Utility

The interpretation of biopsies and aspirates in which atypical hematopoietic cells are identified is often difficult. Malignant diseases can occasionally masquerade as benign processes, while reactive processes may simulate malignancies. The emergence of an understanding of normal and abnormal development of the hematopoietic system and the identification of molecular lesions associated with various malignancies

have enabled development of immunological and molecular probes for the identification of monoclonal populations of lymphocytes and the presence of neoplastic cells of other hematopoietic lineages. Identification of such populations assists significantly in diagnosing leukemia or lymphoma, and/or in detecting its recurrence at levels below those discernible using a light microscope.

2.2 Advantages and Disadvantages

Prudent clinical use of molecular diagnostic methods to identify monoclonal proliferations of lymphoid and myeloid cells, to determine the presence or absence of disease-related chromosomal translocations, or to identify gene sequence alterations requires a thorough understanding of the sensitivity and technical artifacts associated with these methods. In addition, extreme care in assay performance (to avoid carryover of amplification products), and the ability to carefully weigh the results, together with clinical findings and histology, is necessary for diagnosis. To avoid erroneous interpretation, nucleic acid–based hematological assays require careful attention to technical detail and the implementation of rigorous QA measures.

Molecular methods in hematopathology provide the pathologist with tools that can be exquisitely sensitive and specific when properly performed and interpreted. When a neoplastic cell population is defined by the presence of a specific chromosomal translocation or somatic mutation, the neoplastic cells can be sensitively detected due to the absence of the molecular abnormality in normal cells. The same abnormality can be used to detect minimal residual disease (MRD) post-therapy. Quantitative assays can be used to determine effectiveness of therapy and early loss of therapeutic effect.

Limitations of molecular techniques include false-positive and false-negative results for lymphoid clonality assays. The former occurs when there is a clonally restricted immune response that can potentially be misinterpreted as a lymphoid neoplasm, or when the specimen contains a paucity of normal lymphocytes for which one or two may be captured by PCR-based amplification of a surrogate immunoglobulin heavy chain or T-cell receptor (TCR) target (“pseudoclonality”). The latter occurs when the neoplastic cell population comprises a small proportion of cells in a background of normal cells. Also, not all neoplastic lymphoid cells can be detected by the most common amplification-based assays. In general, molecular assays are also limited if a neoplastic population lacks any of the markers that are available for analysis. Conventional cytogenetic studies, and possibly newer microarray-based methods, may be useful in detecting genetic alterations that may be missed using probe-based assays.

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the Centers for Disease Control and Prevention (CDC).[11] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[12]

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

MM05-A2 addresses the QSEs indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section, beginning on page 90.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
						X C28 EP05 EP06 EP09 EP12 EP15 EP17				X	
							GP02	GP02			
		M29				GP29				GP29	
		MM07	MM07		MM07	MM01 MM03 MM06 MM07 MM12 MM13 MM14	MM07			MM03 MM06 MM07 MM14	
MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

MM05-A2 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
X			X	X	X	X	X	X
MM01	MM01	MM01	MM01	MM01	MM01	MM01	MM01	MM01
	MM03	MM03	MM03	MM03	MM03		MM03	
MM06	MM06	MM06	MM06	MM06	MM06	MM06	MM06	
MM07	MM07	MM07	MM07	MM07	MM07	MM07	MM07	MM07
		MM12	MM12	MM12	MM12	MM12	MM12	MM12
	MM13	MM13	MM13					MM13
	MM19	MM19	MM19	MM19	MM19	MM19		

Related CLSI Reference Materials*

C28-A3c **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition (2010).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests. A CLSI-IFCC joint project.

EP05-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP06-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.

EP09-A2-IR **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision) (2010).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

EP12-A2 **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (2008).** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

EP15-A2 **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2006).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.

EP17-A **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits. An NCCLS-IFCC joint project.

GP02-A5 **Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition (2006).** This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the medical laboratory community.

GP29-A2 **Assessment of Laboratory Tests When Proficiency Testing Is Not Available; Approved Guideline—Second Edition (2008).** This document offers methods to assess test performance when proficiency testing (PT) is not available; these methods include examples with statistical analyses. This document is intended for use by laboratory managers and testing personnel in traditional clinical laboratories as well as in point-of-care and bedside testing environments.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

MM01-A2 **Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline—Second Edition (2006).** This document provides guidance for the use of molecular biological techniques for clinical detection of heritable mutations associated with genetic disease.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

MM03-A2 **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufactures and clinical laboratories.

MM06-A2 **Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition (2010).** This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It also presents recommendations for quality assurance, proficiency testing, and interpretation of results.

MM07-A **Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline (2004).** This document addresses FISH methods for medical genetic determinations, identification of chromosomal abnormalities, and gene amplification. Recommendations for probe and assay development, manufacture, qualification, verification, and validation; instrument requirements; quality assurance; and evaluation of results are also included.

MM12-A **Diagnostic Nucleic Acid Microarrays; Approved Guideline (2006).** This guideline provides recommendations for many aspects of the array process including: a method overview; nucleic acid extraction; the preparation, handling, and assessment of genetic material; quality control; analytic validation; and interpretation and reporting of results. A CLSI-IFCC joint project.

MM13-A **Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline (2005).** This document provides guidance related to proper and safe biological specimen collection and nucleic acid isolation and purification. These topics include methods of collection, recommended storage and transport conditions, and available nucleic acid purification technologies for each specimen/nucleic acid type. A CLSI-IFCC joint project.

MM14-A **Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline (2005).** This document provides guidelines for a quality proficiency testing program, including reliable databases; design control in the choice of materials and analytes; good manufacturing processes; documentation procedures; complaint handling; corrective and preventive action plans; and responsive timing of reports. A CLSI-IFCC joint project.

MM19-A **Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline (2011).** This guideline provides comprehensive guidance for planning and implementation of molecular diagnostic testing, including strategic planning, regulatory requirements, implementation, quality management, and special considerations for the subspecialties of molecular genetics, infectious diseases, oncology, and pharmacogenetics.

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