



MM13

Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods

This guideline provides recommendations on proper and safe biological specimen collection and nucleic acid isolation and purification. Topics include collection methods, recommended transport and storage conditions, and available nucleic acid isolation and purification technologies for each specimen and nucleic acid type.

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

Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods

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Abstract

Molecular methods involving nucleic acid hybridization or enzymatic amplification require the isolation and purification of nucleic acids from various biological specimens and microorganisms contained in specimens. CLSI guideline MM13—*Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods* covers the general principles for optimal specimen collection, transport, preparation, and storage for nucleic acid determination for molecular diagnostic testing.

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Foreword

CLSI's molecular diagnostics guidelines discuss the needs of molecular diagnostic testing laboratories and other laboratories that apply molecular methods to the study of nucleic acids in human samples or specimens. MM13, in response to the variety of specimen types, the many variables that can affect examination results, and the extent of molecular test methods, provides general principles for minimizing or eliminating preexamination variables for all molecular tests and specimen types. This guideline is also intended to increase awareness of the specimen handling factors that affect molecular examination results and promote standardization of the preexamination phase of testing.

Since this guideline was first published in 2005, research and development in the preexamination phase of molecular testing has advanced significantly, resulting in new or updated peer-reviewed publications, professional guidance, and international standards. Because the field continues to rapidly evolve, this guideline incorporates the mainstream advancements and best practices that are most appropriate for current clinical work.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM13-A, published in 2005. Several changes were made in this edition, including:

- Providing pertinent information on specimen types in the text instead of in tables
- Adding subchapters on enrichment
- Adding a subchapter on evaluating purified nucleic acids (postextraction)
- Revising and updating subchapters on tissue preparation and cell-free DNA isolation and purification

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

Degradation, disruption, DNA, enrichment, expression, homogenization, RNA, specimen collection, specimen preparation, specimen stability

Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- Terminology information, including:
 - Terms and definitions used in the guideline
 - Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline describes general technical principles for ensuring optimal specimen collection, transport, storage, and nucleic acid isolation for molecular diagnostic test methods. It is intended for all health care professionals responsible for obtaining and transporting specimens from patients or preparing samples for molecular tests. It is also intended for manufacturers of specimen collection devices and sample preparation reagents, kits, and instrumentation.

This guideline also describes specimen collection and transport devices, sample preparation methods and optimal storage conditions, and special precautions for molecular methods. Although this guideline is intended for diagnostic testing, the principles described here may apply to other areas.

MM13 also discusses QMS principles that should be implemented to meet regulatory and accreditation requirements. CLSI documents QMS01¹ and MM19² also provide thorough QMS implementation guidance.

For guidelines and best practices covering specimen collection, transport, handling, and storage safety, refer to CLSI document GP17³ and other available resources (eg, regulatory agencies,⁴ World Health Organization). International guidance is also available.⁵

For specimen handling information unique to specific technologies or subspecialties, consult CLSI documents MM03,⁶ MM05,⁷ MM09,⁸ and I/LA28.⁹

1.2 Background

The expanding discipline of molecular pathology is characterized by the rapid introduction of new disease markers and detection technologies. Nucleic acid targets are isolated from a wide variety of patient specimens, and the targets' quantity and quality are highly affected by specimen collection, handling, and extraction method.

Molecular biological techniques developed for infectious diseases in the last decade enable medical laboratories to:

- Detect the presence or quantity of viruses.
- Determine the microorganism family, genus, or species.
- Determine the viral genotype.

Recently developed genetic tests using purified human DNA detect or identify the presence of somatic variants, as well as the presence of, predisposition to, or carrier status of inherited diseases, such as cystic fibrosis, hereditary hemochromatosis, or Tay-Sachs disease. Because of the inherent instability of RNA, detection of intracellular RNA targets for all molecular testing (from infectious diseases to genetic studies) has lagged behind detection of DNA targets that contribute to patient management. Nevertheless, tests are increasingly becoming available that detect the recurrence of hematological malignancies by quantifying gene translocation fusion transcripts that characterize the disease and appear during or after treatment. RNA's labile nature has made standardizing these tests challenging. A mishandled specimen may produce a negative result due to target degradation rather than the absence of disease.¹⁰

The matrix of variables associated with specimen type, nucleic acid target, and compatibility of sample preparation methods with downstream test methodology is complex. Accordingly, this guideline is organized with these complexities in mind and has been designed for laboratory personnel seeking a comprehensive, easy-to-use reference for molecular specimen handling. Optimal conditions for transport and storage are described, and recommendations for nucleic acid extraction procedures are provided when supported by published studies. In other cases, the experience of committee members or recommendations from manufacturers of commercially available products are used.

Molecular diagnostic methods can themselves be highly variable, so it is important to minimize the preexamination variables associated with specimen collection, transport, processing, and storage. One of this guideline's goals is to advance the standardization of preexamination methods to support the growing list of molecular diagnostic assays, with the important caveat that any of these methods should be optimized or qualified for test systems incorporated into medical diagnostic testing. Laboratories and test developers are encouraged to carefully consider preexamination variables when designing diagnostic test systems and adapt specimen collection, transport, and storage instructions accordingly.

Chapter 5: Preparing Samples for Molecular Testing

This chapter includes:

- Information on different methods used to prepare samples for nucleic acid analysis

A sample is a product derived from the patient specimen. A common specimen type is blood, which is processed (eg, single- or double-spin plasma, peripheral blood mononuclear cells, pellet, whole blood pellet, buffy coat) before nucleic acid extraction. The extracted nucleic acids are then used in various molecular diagnostic test methods. Cell isolation or enrichment methods, along with activation state and cell differentiation stage, affect the quantity and quality of nucleic acids extracted from blood specimens.

5.1 Selecting Commercial Sample Preparation Methods

Best practices for molecular testing should be followed including the use of a unidirectional workflow. Methods should be validated before being applied to patient testing. All molecular methods should be performed using RNase- and deoxyribonuclease (DNase)-free water and molecular grade reagents. (See CLSI document MM19² for more information.)

Nucleic acid extraction can be performed manually or through automated platforms. Important factors to consider before any extraction method is implemented include performance, cost, compatibility with workflow, and laboratory personnel expertise. Laboratories should consider additional factors to evaluate an automated platform.

Factors to consider when a platform is being chosen include the:

- Stated method performance
- Cost to perform the extraction
- Workflow, throughput, and physical laboratory space

5.1.1 Performance

One of the most important factors to consider when the laboratory is choosing a method for nucleic acid extraction is whether the method can reliably yield high quantities of pure nucleic acids from the specimen. There are numerous manual and automated methods to consider. Many manual methods use flow-through columns to improve workflow. Automated platforms are generally either column-based or magnetic bead-based technologies. In medical laboratories, the specimen sources are often varied. Automated platforms can efficiently extract nucleic acids from specimens such as FFPE, sputum, stool, serum, plasma, and whole blood. Some platforms necessitate specimen pretreatment. Some specimen types provide higher nucleic acid yields with a specific extraction technology, specifically when the specimen buffer is incompatible with nucleic acid extraction. Specimen input varies among methods. The volume and amount of nucleic acid yield should align with downstream application input needs.

Chapter 7: Quality System Essentials

This chapter includes information on:

- Facilities and Safety Management
- Process Management
- Documents and Records Management

CLSI document QMS01¹ defines a structured approach to organizing, creating, and maintaining the necessary information for a medical laboratory QMS. It describes 12 quality system essentials (QSEs) that support the laboratory's technical operations, collectively known as the path of workflow. The laboratory's path of workflow consists of preexamination, examination, and postexamination processes, beginning with an order for a laboratory examination and proceeding all the way to provision of the report and archiving of the specimen, as depicted in Figure 2.

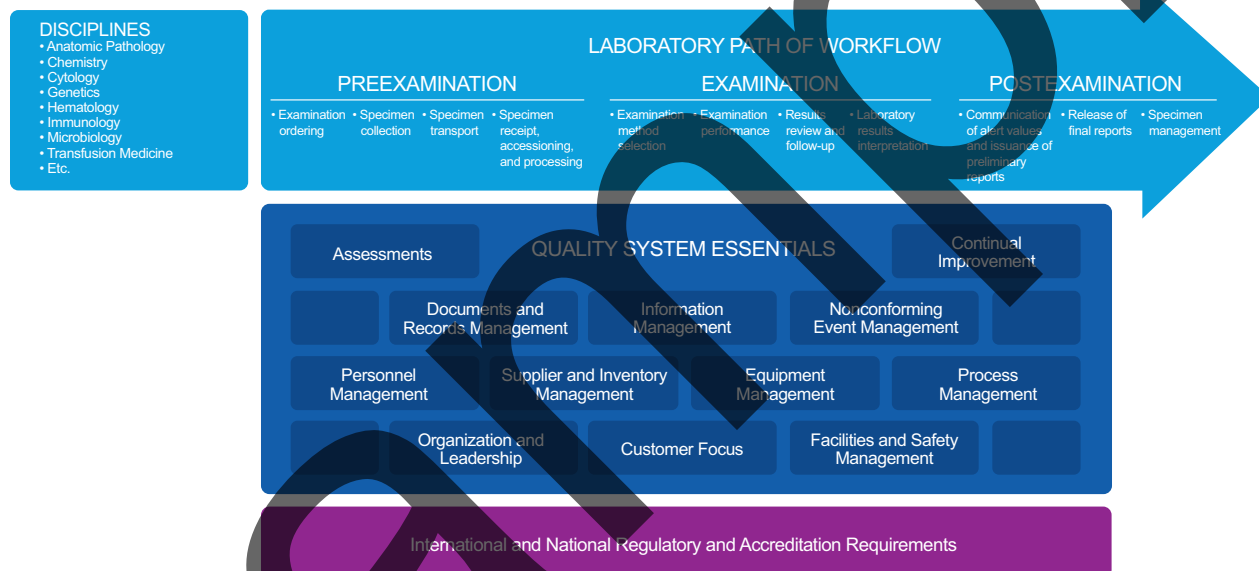


Figure 2. The QMS Model for Laboratory Services (see CLSI document QMS01¹)

QSEs with specific applicability to specimen collection, transport, preparation, and storage include:

- Facilities and Safety Management
- Process Management
- Documents and Records Management

7.1 Facilities and Safety Management

Laboratory services are conducted in facilities that meet applicable requirements for structure and safety (see CLSI document QMS01¹). To avoid contamination of patient specimens, physically separate work areas should be established for specimen

Related CLSI Reference Materials^a

- AUTO02** **Laboratory Automation: Bar Codes for Specimen Container Identification. 2nd ed., 2005.** This document provides specifications for use of linear bar codes on specimen container tubes in the clinical laboratory and for use on laboratory automation systems.
- AUTO12** **Specimen Labels: Content and Location, Fonts, and Label Orientation. 1st ed., 2011.** The purpose of this standard is to reduce human errors currently associated with the lack of standardization of labels on clinical laboratory specimens. The standard identifies the required human-readable elements to appear on specimen labels and specifies the exact locations, fonts, and font sizes of these elements.
- EP23™** **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.
- GP17** **Clinical Laboratory Safety. 3rd ed., 2012.** This document contains general recommendations for implementing a high-quality laboratory safety program, which are provided in a framework that is adaptable within any laboratory.
- I/LA28** **Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays. 2nd ed., 2011.** This document provides guidelines for the development of validated diagnostic, prognostic, and predictive immunohistochemical assays.
- M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** 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.
- MM03** **Molecular Diagnostic Methods for Infectious Diseases. 3rd ed., 2015.** This report 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 manufacturers and clinical laboratories.
- MM05** **Nucleic Acid Amplification Assays for Molecular Hematopathology. 2nd ed., 2012.** 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.
- MM09** **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine. 2nd ed., 2014.** This document addresses diagnostic sequencing using both automated capillary-based sequencers and massively parallel sequencing instruments. Topics include specimen collection and handling; isolation and extraction of nucleic acid; template preparation; sequence generation, alignment, and assembly; validation and verification; ongoing quality assurance; and reporting results.
- MM19** **Establishing Molecular Testing in Clinical Laboratory Environments. 1st ed., 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.

^a 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)

- MM23** **Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms). 1st ed., 2015.** This guideline covers the current state of molecular diagnostic techniques intended for the characterization of solid tumors, and covers a range of clinical applications including diagnosis, prognosis, therapeutic response prediction for available drugs and those still in clinical trials, as well as monitoring and presymptomatic and predisposition testing.
- NBS01** **Blood Collection on Filter Paper for Newborn Screening Programs. 6th ed., 2013.** This document highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper segment of the specimen collection device, and provides instructions on proper specimen handling and transport to ensure quality specimens are consistently obtained for newborn screening analysis.
- POCT07** **Quality Management: Approaches to Reducing Errors at the Point of Care. 1st ed., 2010.** This document presents the core infrastructure for a standardized error tracking system with the primary goals of reducing risk and increasing quality of point-of-care testing, while accumulating standardized data for benchmarking use.
- POCT09** **Selection Criteria for Point-of-Care Testing Devices. 1st ed., 2010.** This document provides guidance on selection of point-of-care testing devices based on the patient care setting and clinical needs. It is designed as an aid to laboratory and facility management to simplify and facilitate the selection process but also allows evaluation of devices to identify those that are optimal to the patient care setting and population served.
- QMS01** **A Quality Management System Model for Laboratory Services. 5th ed., 2019.** This guideline provides a model for medical laboratories to organize the implementation and maintenance of an effective quality management system.
- QMS18** **Process Management. 1st ed., 2015.** This guideline describes four requirements for managing laboratory processes and provides suggestions for effectively meeting regulatory and accreditation requirements, optimizing efficient use of resources, and contributing to patient safety and positive outcomes.

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