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Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline

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

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Lynne Rainen, PhD
Judy C. Arbique, ART
Deshratn Asthana, PhD, MBA, HCLD(ABB)
Marie C. Earley, PhD
Richard L. Geiszler
Frank Krieg-Schneider, PhD
Christine Mannhalter, PhD
Shuji Ogino, MD, PhD
Gary T. Parish
Cynthia Ballas, MT(ASCP)
Laura J. Chandler, PhD
Helen Fernandes, PhD
Maurizio Ferrari, MD
Mirna Lechpammer, MD, PhD
Cindy R. WalkerPeach, PhD
Laurina O. Williams, PhD, MPH

Abstract

Molecular methods involving the hybridization or enzymatic amplification of nucleic acids require the isolation and purification of these nucleic acids from a variety of biological specimens and microorganisms contained in these specimens. CLSI document MM13-A—*Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline* addresses topics that relate 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.

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Foreword

This guideline was developed in response to the exponential growth of the numbers and types of molecular tests performed worldwide and the need to standardize specimen collection and preparation parameters for these tests. Until recently, molecular test methods routinely used by clinical laboratories were largely limited to detection and/or quantitation of viruses or bacteria in only a few specimen types. The accelerated identification of molecular lesions in neoplastic cells; the discoveries of genotypic variations that correlate to disease states; the significance of gene transcription as indicator of disease or response to therapy; and the commercialization of molecular diagnostic tests for routine use have contributed to both the variety of specimens used and the analytical techniques employed.

MM13 is part of a series of guidelines that address the needs of molecular diagnostic testing laboratories or other laboratories that apply molecular methods to the study of nucleic acids in human samples or specimens. Because of the variety of specimen types used, the many variables that can affect test results, and, indeed, the variety of test methodologies employed in molecular laboratories, the Subcommittee on Sample Collection and Handling for Molecular Test Methods concluded that it would be advantageous to provide guidelines that address the general principles for minimizing or eliminating preanalytical variables for all types of molecular tests and for all types of samples. This guideline should increase awareness of the sample handling factors that affect molecular testing results and promote standardization of the preanalytical phase of these test methods.

Key Words

Degradation, disruption, DNA, enrichment, expression, homogenization, RNA

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1 Scope

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

In addition, this document describes specimen collection and transport devices, and sample preparation methods. Optimal storage conditions and special precautions for molecular methods are described. While this document is intended for diagnostic testing, it is possible that the principles described here may apply to other areas.

NOTE: Measurand quality assessment should be undertaken at the level of the test method during method validation and is beyond the scope of this guideline. For information related to measurand (purified DNA or RNA) quality assessment, refer to the most current editions of the following CLSI/NCCLS documents: MM3—*Molecular Diagnostic Methods for Infectious Diseases*; MM5—*Nucleic Acid Amplification Assays for Molecular Hematopathology*; and/or MM9—*Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine*.

2 Introduction

The expanding discipline of molecular pathology is characterized by the rapid introduction of new markers for disease and technologies for their detection. Furthermore, nucleic acid targets must be isolated from a wide variety of clinical specimens, and the quality and quantity of extracted target is highly affected by specimen collection, handling, and choice of extraction method.

Molecular biological techniques developed in the last decade detect the presence or quantity of viruses; determine the family, genus, or species of microorganisms; or determine the viral genotype. Recently developed tests employing purified human DNA enable genetic testing for the presence, predisposition, or carrier status of inherited diseases such as cystic fibrosis, hereditary hemochromatosis, or Tay-Sachs disease to name a few examples. Because of their inherent instability, the measurement of intracellular RNA targets has lagged behind DNA targets in contributing to patient management. Nevertheless, recurrence of hematological malignancies are increasingly detected by quantitation of gene translocation fusion transcripts characterizing the disease and appearing during or subsequent to treatment. The labile nature of RNA in particular has made standardization of these tests difficult or impossible. Furthermore, a negative result in a poorly handled sample may have been due to target degradation rather than the absence of disease.

The Subcommittee on Sample Collection and Handling for Molecular Test Methods recognizes the complex nature of the matrix of variables associated with specimen type, nucleic acid target, and compatibility of sample preparation methods with downstream test methodology. Accordingly, these guidelines are organized with these complexities in mind, and they have been designed for the laboratorian seeking a comprehensive, easy-to-use reference for molecular specimen handling. A chart is provided which allows the user to access specific information by cross-referencing specimen type with the nucleic acid target. Optimal conditions for transport and storage, as well as 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 is used.

Since molecular diagnostic methods can themselves be highly variable, the successful application of these techniques is well served by minimizing the preanalytical variables surrounding specimen acquisition, transport, storage, and processing. It is hoped that this guideline will further the standardization of preanalytical methods for the growing list of clinically valuable molecular diagnostic assays, with the important caveat that any of these methods may require optimization or qualification for test systems incorporated into clinical diagnostic testing. Laboratories and test developers are encouraged to carefully consider preanalytic variables with designing diagnostic tests systems and adapt collection, transport, and storage instructions accordingly.

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 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 U.S. Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol*. 1996;17(1):53-80; and U.S. Department of Health and Human Services (BMBL), CDC, and NIH. *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, 1999, available at: http://www.cdc.gov/od/ohs/biosfty/bmb14/bmb14toc.htm). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*.

4 Definitions

3′ poly(A) tail – a sequence of adenylyl residues at the 3′ end of eukaryotic mRNA; **NOTE:** Almost all mature eukaryotic mRNAs have 3′ poly(A) tails of 40 to 200 nucleotides, those of histones being a notable exception. The poly(A) tail is added enzymatically to the primary transcript, which is first cleaved 10 to 30 nucleotides past a highly conserved AAUAAA sequence. The poly(A) tail is then generated from ATP through the activity of polynucleotide adenylyltransferase. In practical terms, the poly(A) tail on mRNA has facilitated its ready isolation from total cellular RNA by affinity chromatography on oligo(dT) cellulose.

5′ cap – a structural feature present at the 5′ end of most eukaryotic (cellular or viral) mRNA molecules and also some virion mRNA molecules, but not of bacterial mRNA molecules; **NOTE:** It consists of a residue of 7-methylguanosine (m^7G cap) and a triphosphate bridge linking it 5′-5′ to the end of the polynucleotide chain. The cap structure is thought to protect the 5′ end of the mRNA from degradation by phosphatases or nucleases and to facilitate initiation of translation of mRNA by the eukaryotic (but not the bacterial) ribosome.

analyte – component represented in the name of a measurable quantity (ISO 17511)[1]; **NOTE 1:** In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte. In both cases, the long phrase represents the measurand (ISO 17511)[1]; **NOTE 2:** In the type of quantity "catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma," "lactate dehydrogenase isoenzyme 1" is the analyte. The long first phrase designates the measurand (ISO 18153)[2]; **NOTE 3:** The analyte is the particular component of interest to the patient.

Related CLSI/NCCLS Publications*

LA4-A4 **Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Fourth Edition (2003).** This document addresses the issues associated with specimen collection, the filter paper collection device, and the transfer of blood onto filter paper, and provides uniform techniques for collecting the best possible specimen for use in newborn screening programs.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on U.S. 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.

MM3-P2 **Molecular Diagnostic Methods for Infectious Diseases; Proposed Guideline—Second Edition (2005).** 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 manufacturers and clinical laboratories.

MM5-A **Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline (2003).** This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse-transcriptase polymerase chain reaction (RT-PCR) techniques and includes information on specimen collection, sample preparation, test reporting, test validation, and quality assurance.

MM9-A **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline (2004).** This document addresses automated, PCR-based, dideoxy-terminator, and primer extension sequencing done on gel- or capillary-based sequencers. Topics covered include: specimen collection and handling; isolation of nucleic acid; amplification and sequencing of nucleic acids; interpretation and reporting of results; and quality control/assessment considerations as appropriate.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

