CLINICAL AND LABORATORY STANDARDS INSTITUTE®

January 2008

H21-A5

Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition

This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storing plasma for coagulation testing; and general recommendations for performing the tests.

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

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement, but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advancements in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential, and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeals Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeals, documented in the CLSI Standards Development Policies and Processes, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

H21-A5
Vol. 28 No. 5
ISBN 1-56238-657-3 Replaces H21-A4
ISSN 0273-3099 Vol. 23 No. 35

Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition

Volume 28 Number 5

Dorothy M. Adcock, MD
Daniel M. Hoefner, MT, PhD
Kandice Kottke-Marchant, MD, PhD
Richard A. Marlar, PhD
Diane I. Szamosi, MA, MT(ASCP), SH(ASCP)
David J. Warunek, PhD, MBA

Abstract

Clinical and Laboratory Standards Institute H21-A5—*Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition* is an update of the previous edition published in 2003. The guideline provides procedures for the collection, transport, and processing of blood specimens for plasma-based and molecular coagulation testing. Tests of the coagulation system are very sensitive to storage (time and temperature), concentration of anticoagulant, and surface of containers; attention to these parameters is important. H21-A5 is primarily directed toward laboratory and/or clinical personnel responsible for obtaining patient specimens and preparing samples for plasma-based or molecular coagulation testing.

Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition*. CLSI document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2008.

Suggested Citation

CLSI. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition.* CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Proposed Guideline
September 1980

Tentative Guideline
January 1982

Approved Guideline—First Edition
December 1986

Approved Guideline—Second Edition
December 1991

Approved Guideline—Third Edition
December 1998

Approved Guideline—Fourth Edition
December 2003

Approved Guideline—Fifth Edition
January 2008

ISBN 1-56238-657-3
ISSN 0273-3099

Contents

Foreword

Because of the many variables that can affect coagulation test results, CLSI has made available this guideline, which describes procedures for collection, transport, preparation, and storage of samples for plasma-based coagulation assays and molecular hemostasis testing. This publication should enhance the uniformity of sample collection, preparation, and handling and, thereby, reduce many of the preanalytical variables that can affect the test results.

This document replaces the fourth edition of the approved guideline, H21-A4, which was published in 2003. Several changes were made in this edition; chief among them is the revision of transportation and storage guidelines for plasma-based hemostasis testing and the addition of information pertinent to the collection, transportation, and processing of specimens for molecular hemostasis assays.

Key Words

Activated partial thromboplastin time, citrate, coagulation, preanalytical variables, prothrombin time, sample storage, specimen collection, specimen transport

Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition

1 Scope

This guideline covers the procedures for the collection, transport, and processing of specimens for plasma-based coagulation and molecular hemostasis tests. Many variables, including anticoagulant volume and concentration, type of tube additive, duration and temperature of specimen storage, and surface of containers used for specimen collection and storage, may affect plasma-based coagulation test results. The reliability and accuracy of molecular test results also depend upon a variety of specimen collection, transport, and storage factors. The molecular testing in this document refers to DNA testing only.

The document is directed toward laboratory and/or clinical personnel responsible for obtaining and preparing patient specimens and for plasma-based coagulation and molecular hemostasis testing. It is also aimed at manufacturers of products involved in specimen collection, storage, preparation, and testing of plasma-based or molecular hemostasis assays. This document does not address whole blood clotting tests, platelet function tests, or point-of-care testing. H21-A5 does not provide general guidelines for the performance of coagulation testing. Performance guidelines for specific coagulation assays are addressed in other CLSI documents, such as those for PT and APTT assays (ie, H47[1]) and fibrinogen assay (ie, H30[2]).

2 Introduction

A procedural guideline for the collection, transport, and processing of specimens for plasma-based coagulation and molecular hemostasis tests is necessary, as many preanalytical variables may affect test results (eg, concentration and volume of anticoagulant or additive; specimen and sample storage time and temperature). Because important diagnostic and therapeutic decisions are based on the results of hemostasis assays, a procedural guideline for the collection, transport, and processing of specimens for the general performance of plasma-based coagulation and molecular hemostasis assays is warranted.

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 US Centers for Disease Control and Prevention.[3] 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 CLSI document M29.[4]

4 Definitions

activated partial thromboplastin time (APTT) – the time, in seconds, required for a fibrin clot to form in a plasma sample after appropriate amounts of calcium chloride, and a partial thromboplastin reagent (phospholipid plus a contact activator), are mixed with the sample; **NOTE:** The APTT measures the intrinsic and common coagulation pathways.

anticoagulant – an agent that prevents the coagulation of blood.

blood collection device – a capped tube that contains a vacuum (otherwise known as an evacuated tube) usually held by an adaptor with attached needle, syringe, or other device with a nonactivating surface used to collect a blood sample with the use of a needle assembly.

blood collection system – a system consisting of several components, such as catheter, connecting device, syringe, needle, and collection device, used for blood collection.

coagulation factors – the various components of the blood coagulation system; **NOTE:** The following factors (including synonyms which are, or were in use) are known:

Factor I (fibrinogen)
Factor II (prothrombin)
Factor III (commonly termed thromboplastin, tissue factor)
Factor IV (commonly termed calcium)
Factor V (labile factor)
Factor VII (stable factor)
Factor VIII (antihemophilic factor [AHF], antihemophilic globulin [AHG], antihemophilic factor A, Factor VIII:C)
Factor IX (plasma thromboplastin component [PTC], Christmas factor, antihemophilic factor B)
Factor X (Stuart factor, Prower factor, Stuart-Prower factor)
Factor XI (plasma thromboplastin antecedent [PTA], antihemophilic factor C)
Factor XII (Hageman factor, surface factor, contact factor)
Factor XIII (fibrin stabilizing factor [FSF], fibrin stabilizing enzyme, fibrinase)
Other factors: (prekallikrein [Fletcher factor], high molecular weight kininogen [Fitzgerald factor]).

container – the receptacle that contains the specimen.

dead space volume – the volume of blood that would fill the length of a catheter lumen; **NOTE:** This term is used in the collection of blood from indwelling vascular access devices.

deoxyribonucleic acid (DNA) – a type of nucleic acid; a polynucleotide having a specific sequence of deoxyribonucleotide units principally serves as the carrier of genetic information.

International Normalized Ratio (INR) – patient's prothrombin time (PT) test result expressed as a ratio to the mean normal prothrombin time (MNPT) standardized (or normalized) for the potency of the thromboplastin used in the assay (revised from ISO/DIS 17593)[5]; **NOTE:** INR = (Plasma PT÷MNPT)^{ISI}.

International Sensitivity Index (ISI) – a quantitative measure, in terms of the first International Reference Preparation of thromboplastin, human, combined, coded 67/40, of the responsiveness of a prothrombin-time system to the defect induced by oral anticoagulants (WHO 880).[6]

nonactivating surface – a surface that minimizes activation of anticoagulated whole blood specimens/plasma samples and results in the inhibition of platelet and coagulation factor activation (as indicated by lengthening or shortening of the PT or APTT).[7,8]

prothrombin time (PT) – time in seconds required for a fibrin clot to form in a plasma sample after optimal amounts of tissue thromboplastin (tissue factor plus phospholipid) and calcium chloride are added to the sample; **NOTE 1:** The PT measures the extrinsic and common coagulation pathways; **NOTE 2:** WHO defines PT in the following way: *PT (tissue-factor-induced coagulation time)*—the clotting time of a plasma (or whole blood) sample in the presence of a preparation of thromboplastin and the appropriate

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 approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.* 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:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External & Internal	Facilities & Safety

H21-A5 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 on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
GP2				H3	X C28 H1 H3 M29 MM1 MM5 MM13	GP2 H1		MM5			H3 M29

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

H21-A5 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
H3 MM1 MM5	X H1 H3 H51 MM1 MM5 MM13	X H3 H51 MM1 MM13	X H3 H30 H47 H51 MM1 MM5 MM13	H30 H47 H51 MM1 MM5	H30 H47 H51 MM1 MM5	H51 MM1 MM5	MM1 MM5	MM1 MM5 MM13

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.*

Related CLSI Reference Materials*

C28-A2 **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (2000).** This document provides guidance for determining reference values and reference intervals for quantitative clinical laboratory tests.

GP2-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.

H1-A5 **Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003).** This document contains requirements for venous blood collection tubes and additives, including technical descriptions of ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin compounds used in blood collection devices.

H3-A6 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition (2007).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

H30-A2 **Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition (2001).** This document provides general guidelines for performing the fibrinogen assay in the clinical laboratory. It also includes reporting of results and *in vivo* and *in vitro* conditions that may alter results.

H47-A **One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline (1996).** This document provides guidelines for performing the PT and APTT tests in the clinical laboratory, for reporting results, and for identifying sources of error.

H51-A **Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity; Approved Guideline (2002).** This guideline describes the following: appropriate test specimens; reagents and materials; methods of platelet agglutination and ELISA; preparation of reference curves; determination of reference intervals; quality control procedures; result interpretation; and sources of error for assays of von Willebrand factor antigen and ristocetin cofactor activity. A brief description of von Willebrand disease and its various subtypes is included, as well as a list of references to more comprehensive reviews of this commonly inherited and rarely acquired bleeding disorder.

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.

MM1-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.

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.

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.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Explore the Latest Offerings From CLSI!

As we continue to set the global standard for quality in laboratory testing, we are adding products and programs to bring even more value to our members and customers.

By becoming a CLSI member, your laboratory will join 1,600+ other influential organizations all working together to further CLSI's efforts to improve health care outcomes. You can play an active role in raising global laboratory testing standards—in your laboratory, and around the world.

Find out which membership option is best for you at **www.clsi.org/membership.**

Find what your laboratory needs to succeed! CLSI U provides convenient, cost-effective continuing education and training resources to help you advance your professional development. We have a variety of easy-to-use, online educational resources that make *e*Learning stress-free and convenient for you and your staff.

See our current educational offerings at **www.clsi.org/education.**

When laboratory testing quality is critical, standards are needed and there is no time to waste. eCLIPSE™ Ultimate Access, our cloud-based online portal of the complete library of CLSI standards, makes it easy to quickly find the CLSI resources you need.

Learn more and purchase eCLIPSE at **clsi.org/eCLIPSE.**

For more information, visit www.clsi.org today.

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: 610.688.0100 **Toll Free (US):** 877.447.1888 **F:** 610.688.0700

E: customerservice@clsi.org **www.clsi.org**

ISBN 1-56238-657-3