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

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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

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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.

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**Suggested Citation**


**Proposed Guideline**

- **Approved Guideline—Third Edition**
  - September 1980
  - December 1998

**Tentative Guideline**

- **Approved Guideline—Fourth Edition**
  - January 1982
  - December 2003

**Approved Guideline—First Edition**

- **Approved Guideline—Fifth Edition**
  - December 1986
  - January 2008

**Approved Guideline—Second Edition**

- December 1991

ISBN 1-56238-657-3
ISSN 0273-3099
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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, H47³) and fibrinogen assay (ie, H30²).

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 M29.⁵

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, Proctor factor, Stuart-Proctor 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); **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).

**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).

**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
- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Information Management
- Process Control
- Occurrence Management
- Assessments—External & Internal
- Process Improvement
- Customer Service
- 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.

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.

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


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.


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 Occationally 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.


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.

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