CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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H47-A2

One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition

This document provides guidelines for performing the PT and APTT tests in the clinical laboratory, for reporting results, and for identifying sources of error.

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

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One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document H47-A2—*One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition* describes the principles and procedures necessary for the routine performance of the PT and APTT by conventional techniques using citrated plasma. Each of the two tests measures the time for a fibrin clot to develop in test plasma after activation. The chemical reactions are complex and, characteristically, results are affected by preexamination (preanalytical) and examination (analytical) variables. The PT and APTT are important screening tests used in laboratory evaluation of patients suspected to have disorders of blood coagulation, including the presence of circulating coagulation inhibitors. The PT measures the extrinsic or tissue factor pathway of the coagulation system and is used to monitor oral anticoagulant therapy. The APTT measures the intrinsic coagulation pathway and is used in monitoring heparin therapy. The objective of this guideline is to improve test reproducibility through standardization of technique and ensure clinical relevance by setting test performance goals. The document also highlights the international effort for standardization of the PT through the use of the international normalized ratio (INR).

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Foreword

Since its original description by Quick[1] in 1935, the prothrombin time (PT) has remained an important screening test in the laboratory evaluation of patients with suspected disorders of blood coagulation. It is the most common coagulation test performed in the clinical laboratory. Although the PT was originally described as a specific, one-stage assay of prothrombin or Factor II, it is sensitive to quantitative or qualitative abnormalities of any of the factors involved in the extrinsic and common pathways of the coagulation system (Factors II, V, VII, X, and fibrinogen), as well as inhibitors of these factors. It is an indicator of moderate to severe hepatic disease or chronic hepatic disease. The PT is also the most commonly used test for monitoring antivitamin K therapy.

Thromboplastin, a phospholipid/tissue factor preparation and the principal reagent used in the PT assay, is commercially available in a variety of preparations of human or animal origin, or human or animal recombinant material. There are differences among commercial thromboplastin preparations in their responsiveness to reductions in coagulation factors that may affect their usefulness, particularly in the monitoring of antivitamin K therapy.[2-6]

The activated partial thromboplastin time (APTT) is sensitive to quantitative and qualitative abnormalities in the intrinsic and common pathways of coagulation. It is the second most common coagulation procedure performed in routine laboratories. The APTT is particularly sensitive to defects of the intrinsic coagulation pathway (Factors VIII, IX, XI, XII, prekallikrein, and high molecular weight kininogen).[7,8] It is commonly used for monitoring unfractionated heparin anticoagulant therapy. It detects other types of pathological inhibitors of blood coagulation, the most common of which is the lupus anticoagulant (LA), and it is used to monitor factor replacement therapy. APTT reagents are a mixture of procoagulant phospholipids and a contact activator. The phospholipids may be of human, animal, or vegetable origin, and there are a variety of activating substances (eg, celite, kaolin, micronized silica, ellagic acid).

Ideally, the APTT is prolonged when levels of coagulation factor activity fall below the 95% confidence limit of the reference interval. However, a number of studies have shown considerable differences in the responsiveness of the various APTT reagents to mild and moderate factor deficiencies, particularly deficiencies of Factor VIII and/or Factor IX.[7-10] A similarly variable sensitivity of the APTT to circulating LAs has been reported.[11] Likewise, marked APTT variability in responsiveness to heparin has been observed among commercially available APTT reagents.[8,12]

This document is written for laboratory and/or clinical personnel responsible for the performance, quality control, and reporting of the PT and APTT tests, as well as for manufacturers of coagulation instruments and reagents who are responsible for maintaining appropriate performance standards. This document should be used in conjunction with CLSI documents H54 and H57.[13,14]

H47-A2 provides guidelines for the routine performance of the PT and APTT by conventional techniques using citrated plasma. Because both tests are strongly affected by a variety of preexamination and examination variables, adherence to the recommended techniques will improve precision and accuracy among laboratories. Recommendations on result reporting and safety precautions are provided. This document replaces the first edition approved guideline, H47-A, which was published in 1996. Several changes were made in this edition; chief among them is the addition of information related to the following:

- validating and calibrating PT reagents;
- local system calibration;
- PT mixing studies;
- APTT mixing studies;
- monitoring direct thrombin inhibitors;

- establishing heparin therapeutic ranges; and
- factor sensitivity determination.

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all challenges to harmonization. In light of this, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, the term *accuracy,* in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects. *Trueness* is used in this document when referring to the "closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand"; the measurement of trueness is usually expressed in terms of *bias. Precision* is defined as the "closeness of agreement between independent test/measurement results obtained under stipulated conditions." As such, it cannot have a numerical value, but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the "dispersion of results of measurements obtained under specified conditions." In addition, different components of precision are defined in H47-A2, primarily *repeatability,* ie, "the closeness of the agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement"; while *reproducibility* describes "the closeness of agreement of results of measurements under changed conditions."

Key Words

Activated partial thromboplastin time (APTT), citrate, coagulation, coagulation factor(s), control (plasma), fibrinogen, international sensitivity index (ISI), international normalized ratio (INR), phospholipids, prothrombin time (PT), thrombin time, thromboplastin, tissue factor

One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition

1 Scope

This document gives general guidelines for performing the prothrombin time (PT) and activated partial thromboplastin time (APTT) by a conventional routine method using citrated, platelet-poor plasma. H47 does not deal with alternative methods using citrated whole blood, capillary blood obtained by the fingerstick method, or nonclotting-based end-point detection, such as chromogenic substrate assay.

2 Introduction

The results of the PT and APTT tests can be affected by a number of preexamination variables, such as method of blood collection; surface characteristics of collection containers; type and concentration of anticoagulant; specimen and sample storage conditions; and examination variables, such as sample incubation time and temperature, contact activation time, type of reagents, and the method of end-point detection. In this document, standard methods for collection, transport, and processing of blood specimens are referenced in CLSI document H21,[15] and test performance specifications are described. This is intended to minimize the effects of such variables, improve precision and accuracy, and, thus, the clinical usefulness of the PT and APTT.

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.[16] 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.[17]

4 Terminology

4.1 Definitions

In this publication, the following definitions of terms are used:

calibration – set of operations that establishes, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards (VIM93)[18]; **NOTE 1:** According to the US Code of Federal Regulations, calibration is the process of testing and adjustment of an instrument, kit, or test system, to provide a known relationship between the measurement response and the value of the substance being measured by the test procedure (42 CFR § 493.1217)[19]; **NOTE 2:** The term is sometimes used to describe different situations; **NOTE 3:** See **calibration line** and **direct INR determination** below.

calibration line – the graphic relationship (typically linear) between the clotting time in seconds and the INR of certified plasmas.

certified plasmas – normal or abnormal plasma samples assigned a PT/INR value by a manufacturer or reference center, using a manual method based on World Health Organization (WHO) accepted protocol determined against an appropriate thromboplastin IRP (or manufacturer or standard reference reagent) that has been calibrated against the appropriate WHO standard in a multicenter study (ie, a minimum of three laboratories for a primary standard and two laboratories for a secondary standard); **NOTE:** See **standard reference reagent** below.

coagulation factor – one of a group of components of blood plasma that interact to form a blood clot.

contact activator – a substance that activates coagulation Factor XII to active proteolytic enzyme; **NOTE:** These activators are normally negatively charged particulate substances but may be soluble compounds.

control material//**control** – a device, material, solution, or lyophilized preparation intended for use in the quality control process; **NOTE 1:** The expected reaction or concentration of analytes of interest are known within limits ascertained during preparation and confirmed in use; **NOTE 2:** Control materials are generally not used for calibration in the same process in which they are used as controls.

direct INR determination – INR determination from a PT/INR calibration line determined using certified plasmas without employing an ISI and mean normal prothrombin time (MNPT).

direct thrombin inhibitor – a class of drugs (either oral or intravenous [IV]) that directly inhibit the enzyme thrombin (without the need for a cofactor).

generic ISI – an ISI determined for a thromboplastin that is not instrument-specific (ie, determined for a group of instruments that uses the same general method for end-point detection, such as manual, photo-optical, or mechanical methods); **NOTE:** See **international sensitivity index** and **thromboplastin-specific/instrument-specific ISI** below.

heparin – a polysaccharide characterized by its anticoagulant properties; **NOTE:** There are a variety of heparin "types," which have different affects on the APTT and PT coagulation tests. **Unfractionated heparin (UFH)** is a class of IV drugs that indirectly (through antithrombin) inhibit the enzymes thrombin and to some extent, factor Xa. The APTT is proportionately prolonged in the presence of UFH. **Low molecular weight heparin (LMWH)** is a class of heparin drugs consisting of smaller, more uniform-sized heparin molecules that inhibit mainly factor Xa, and minimally, and not in a dose-dependent manner, affect the APTT. **Pentasaccharide** is a heparin analog of five heparin subunits that inhibits factor Xa and only marginally affects the APTT.

international normalized ratio (INR) – the patient's PT test result expressed as a ratio to a normal population (MNPT), which has been standardized (or normalized) for the potency of the thromboplastin used in the assay (revised from ISO/CD 17593)[20]; **NOTE:** INR = $(\text{Plasma PT} \div \text{MNPT})^{\text{ISI}}$.

international reference preparation (IRP) – a thromboplastin with defined biological activity used to calibrate other reference preparations and secondary or manufacturer's standards; **NOTE 1:** There are three species of IRP: bovine, rabbit, and human, which can be produced from original biological sources or other recombinant sources; **NOTE 2:** IRPs can only be used in combination with the manual technique (tilt-tube method or other methods that have been validated); **NOTE 3:** WHO and European Union certified reference material (CRM) standards are examples of IRPs; **NOTE 4:** IRPs are also sometimes referred to as primary standards.

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

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

H47-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 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
			H57		X C03 C28 H21 H54 H57 M29			H57			M29

Adapted from CLSI/NCCLS document HS01—*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.

H47-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
	H21	H21	X	X	X H57			

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

C03-A4 **Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline—Fourth Edition (2006).** This document provides guidelines on water purified for clinical laboratory use; methods for monitoring water quality and testing for specific contaminants; and water system design considerations.

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

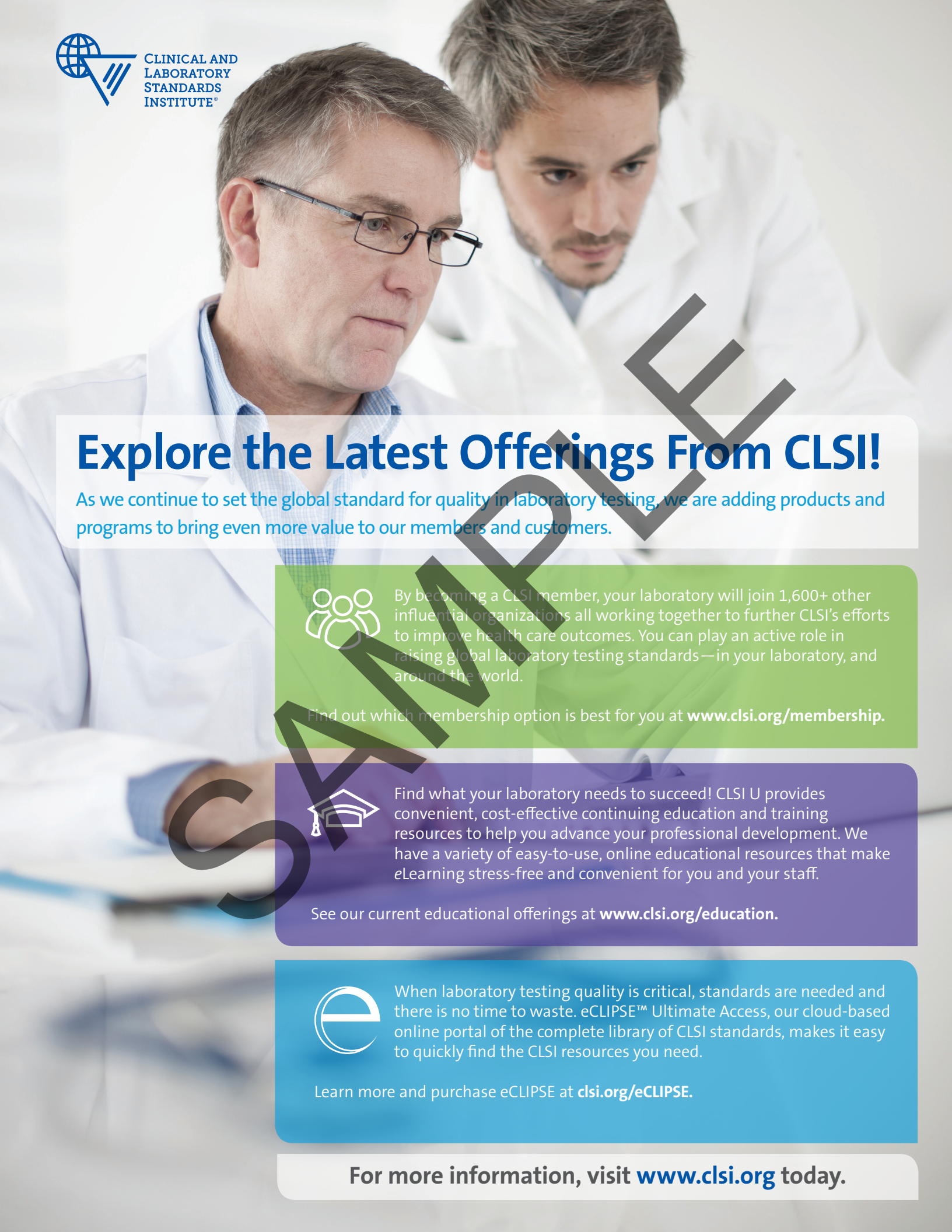
H21-A5 **Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition (2008).** This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storing plasma for coagulation testing; and general recommendations for performing the tests.

H54-A **Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline (2005).** This document describes the use of certified plasmas to enhance performance of the prothrombin time (PT)/International Normalized Ratio (INR) system test; reviews limitations of the INR system that may occur when a manufacturer-determined ISI is used without local verification or calibration; and provides a rationale for performing local ISI verification with recommendations as to when PT calibration may be indicated. Part I is a detailed, expanded account for manufacturers and Part II is an abbreviated version useful for the clinical laboratory.

H57-A **Protocol for the Evaluation, Validation, and Implementation of Coagulometers; Approved Guideline (2008).** This document provides guidance and procedures to the end user and manufacturer for the selection, evaluation, validation, and implementation of a laboratory coagulometer.

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.

* 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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