CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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H54-A

Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline

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.

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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Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document H54-A—*Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline* is one in a series of guidelines that addresses methodology in blood coagulation testing. It is intended to provide guidance for both manufacturers and clinical laboratory personnel responsible for reporting patient INR results. H54 describes the use of certified plasmas to enhance performance of the prothrombin time (PT)/International Normalized Ratio (INR) system test; reviews limitations of the PT/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. This guideline is published in two parts. Part I provides an expanded account of the subject and Part II is an abbreviated version that may be useful in the clinical laboratory. Methods of calculating local ISI are provided and the procedure for creating a calibration line for direct INR determination is included. In the expanded guideline, the method of certified plasma preparation and method of INR value assignments are also described in detail. This guideline includes a recommended INR range that certified plasmas should cover and recommended number of certified plasmas required for local ISI calibration. A protocol for performing calibration of PT systems is provided. The objective of this guideline is to improve precision and trueness (accuracy) of PT/INR systems and enhance both laboratory standardization and patient care.

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Foreword

The prothrombin time (PT) is the most widely performed coagulation assay. It is commonly used to monitor antivitamin K therapy (AVK). The wide use of the PT has resulted in the introduction of numerous thromboplastin reagents and coagulation instruments. Thromboplastin reagents can vary widely in their response to AVK therapy depending on the source material from which they are derived. This can result in a wide range of PT results on identical patient plasmas. Patient samples have shorter clotting times when less responsive PT reagents are used and longer clotting times when a more responsive reagent is used. A wide assortment of instruments is available to measure PT assays using a number of different technologies for endpoint detection (e.g., optical-based or mechanical clot detection). PT test results may vary depending on the clot detection mechanism employed and brand of coagulation analyzer used. Variation in both thromboplastin reagent and instrumentation has contributed to a lack of standardization in PT test results.

In 1983, the World Health Organization (WHO) introduced the International Normalized Ratio (INR) for PT reporting in an effort to offset variation in thromboplastin reagent responsiveness and enhance standardization. An International Sensitivity Index (ISI) is assigned to each commercial lot number of thromboplastin reagent. The ISI is a measure of a reagent's responsiveness to depressed functional levels of vitamin K-dependent coagulation factors compared to the primary WHO International Reference Preparation (IRP). The INR is a mathematical conversion of the PT calculated as follows:

$$INR = (\text{Plasma PT} \div \text{MNPT})^{ISI}.$$

Though the INR system has improved PT reporting, it is still associated with unexpectedly high degrees of inconsistency in values between laboratories and significant variation in locally reported INRs, compared to expected or true results. Such variation may negatively impact patient care as inaccuracies in INR determination can result in chronic over- or under-anticoagulation, resulting in increased patient morbidity and mortality.

Causes for variation in INR include, but are not limited to: 1) incorrect determination of the mean normal prothrombin time (MNPT) or PT; 2) difference in sodium citrate concentration employed for ISI determination compared to that used locally; 3) local effect of the reagent/instrument combination on the manufacturer's assigned ISI; 4) incorrect ISI value applied locally; 5) incorrect choice of IRP for reagent calibration, causing an inaccurate ISI value; and 6) inaccuracy and imprecision in the calibration of the commercial reagent against the appropriate IRP, causing an inaccurate and imprecise ISI value.

In order to optimize performance of the PT, it is recommended that a thromboplastin with an assigned ISI value specific for the laboratory's thromboplastin/instrument combination be used. This is in preference to a thromboplastin lacking an instrument-specific ISI (generic ISI [see the Definitions section]). When using a reagent with a thromboplastin/instrument-specific ISI, local ISI verification should be performed to ensure the ISI value is correct for the laboratory. If different, local calibration should be performed. When using a generic ISI, local verification is mandatory and local ISI calibration is strongly recommended.

Local PT calibration has been demonstrated to enhance the trueness (accuracy) and interlaboratory precision of INR determination. Local application of a conventional WHO calibration methodology is not feasible, as it is a labor-intensive, costly procedure using the manual tilt-tube method, and there is insufficient IRP available for individual laboratories. Instead, certified plasma samples with assigned PT/INR values can be used in individual laboratories to validate and if needed, calibrate the local instrument/reagent system. Local calibration can be achieved using certified plasmas by either calculating an ISI locally using orthogonal regression or by generating a calibration line upon which INR values are interpolated, a direct INR.

With the use of certified plasmas for INR verification and/or calibration of the local PT/INR systems, it is anticipated that clinical laboratories will not only report more accurate INRs, but also enhance laboratory to laboratory consistency, leading to improvements in monitoring antivitamin K therapy and, ultimately, patient outcomes.

A Note on Terminology

Clinical and Laboratory Standards Institute (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 obstacles to harmonization. Despite these obstacles, CLSI recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In keeping with CLSI's commitment to align terminology with that of ISO, the following describes the metrological terms and their uses in H54-A:

The term *accuracy* 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." *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 H54-A, primarily *repeatability* (i.e., "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." The CLSI Harmonization Policy recognizes ISO terms as the preferred terms. When appropriate, alternative terms are included parenthetically to help avoid confusion.

Users of H54-A should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in the Definitions section of this guideline.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

Key Words

Calibration, certified plasmas, international normalized ratio (INR), international sensitivity index (ISI), prothrombin time (PT), thromboplastin, verification

Acknowledgment

This guideline is being developed through the cooperation of the CLSI Area Committee on Hematology and its Subcommittee on Calibration Plasmas, and Technical Committee C5, Haemostaseology, of the Department for Medical Standards (Normenausschuss Medizin) at the German Standards Institute (Deutsches Institut für Normung [DIN]). Representatives of both CLSI and DIN are participating in the development of each organization's respective standard. It is expected that this effort will advance the international harmonization of this important hematology guideline, thereby improving healthcare delivery worldwide. The DIN representative for this project is Heinz Beeser, MD, PhD, Institute for Quality Management and Standardization in Transfusion Medicine, Teningen, Germany.

Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline

1 Scope

CLSI document H54 reviews limitations of the INR system and provides a rationale for performing local PT/INR verification with recommendations as to when calibration may be indicated. The method of certified plasma preparation and method of INR value assignments are described in detail. Also included are the recommended INR range that certified plasmas should cover and recommended number of certified plasmas required for local ISI calibration. A protocol for performing calibration of PT systems is provided. Methods of calculating local ISI are included, as well as the procedure for creating a calibration line in order to interpolate a direct INR.

To facilitate improved precision and accuracy of PT assay results, enhance laboratory standardization, and thereby optimize patient results and care, the guideline has been divided into two parts. Though both may be educational for all users, Part I is primarily intended for manufacturers of the reagents and instruments used in the PT/INR system, and for manufacturers of material such as certified plasmas to standardize the PT assay; Part II is written for laboratory professionals responsible for the performance of prothrombin time (PT) assays.

2 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;17:53-80). 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*.

3 Definitions

accuracy (of measurement) – closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)[1]; **NOTE:** See **measurand** and **trueness**, below.

antivitamin K (AVK) plasma – plasma from an individual on antivitamin K (AVK) therapy; **NOTE:** See **vitamin K antagonist**, below.

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)[1]; **NOTE 1:** According to the U.S. 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)[2]; **NOTE 2:** The term is sometimes used to describe different situations; **NOTE 3:** See **calibration line** and **direct INR determination**, below.

The Quality System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality 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 system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any healthcare service's path of workflow (i.e., 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 quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

H54-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
				H1	X C28 H3 H21						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.

H54-A 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/NCCLS Publications section on the following page.

Preexamination					**Examination**		**Postexamination**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
				H47	H47			

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

Related CLSI/NCCLS Publications*

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.

H1-A5 **Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003).** This standard contains requirements for blood collection tubes and additives including heparin, EDTA, and sodium citrate.

H3-A5 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.

H21-A4 **Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition (2003).** This guideline contains procedures for collecting, transporting, and storing blood; processing blood specimens; storing plasma for coagulation testing; and general recommendations for performing the tests.

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.

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.

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

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