

June 1996

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



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



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

Abstract

This document is a consolidation of the following previously published documents:

- H28-T, *One-Stage Prothrombin Time Test (PT); Tentative Guideline.*
- H29-T, *Activated Partial Thromboplastin Time Test; Tentative Guideline.*

One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline (NCCLS document H47-A) is part of a series of guidelines that addresses methodology in blood coagulation. H47-A also responds to comments on the two constituent documents. It 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 pretest and analytic variables. The PT and APTT are important screening tests to be 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 it is used to monitor oral anticoagulant therapy. The APTT measures the intrinsic coagulation pathway and it is used in monitoring heparin therapy. The objective of this guideline is to improve test reproducibility through standardization of technique and to 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).

[NCCLS. *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test ; Approved Guideline.* NCCLS document H47-A (ISBN 1-56238-301-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1996.]

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H47-A
ISBN 1-56238-301-9
ISSN 0273-3099

June 1996

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Volume 16 Number 3

Charles F. Arkin, M.D.
E.J. Walter Bowie, M.D.
James J. Carroll, Ph.D.
H. James Day, M.D.
J. Heinrich Joist, M.D., Ph.D.
Jane G. Lenahan
Richard A. Marlar, Ph.D.
Douglas A. Triplett, M.D.



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Suggested Citation

NCCLS. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline. NCCLS document H47-A (ISBN 1-56238-301-9). NCCLS, 940 West Valley Road, Wayne, Pennsylvania 19087, 1996.

Proposed Guideline

September 1982

Tentative Guideline

December 1992

Approved Guideline

June 1996

ISBN 1-56238-301-9

ISSN 0273-3099

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Committee Membership

Area Committee on Hematology

Eugene L. Gottfried, M.D.
Chairholder

San Francisco General Hospital
San Francisco, California

Subcommittee on Coagulation

Charles F. Arkin, M.D.
Chairholder

Boston University Medical Center
Boston, Massachusetts

E.J. Walter Bowie, M.D.

Mayo Clinic
Rochester, Minnesota

James J. Carroll, Ph.D.

Ortho Diagnostic Systems, Inc.
Raritan, New Jersey

H. James Day, M.D.

Temple University Hospital
Philadelphia, Pennsylvania

J. Heinrich Joist, M.D., Ph.D.

St. Louis University Medical Center
St. Louis, Missouri

Jane G. Lenahan

Medical Laboratory Automation, Inc.
Florham Park, New Jersey

Richard A. Marlar, Ph.D.

University of Colorado Health Sciences Center
Denver, Colorado

Douglas A. Triplett, M.D.

Ball Memorial Hospital
Muncie, Indiana

Advisors

John T. Brandt, M.D.

Ohio State University Medical Center
Columbus, Ohio

C. Harold Mielke, Jr., M.D., F.A.C.P.

Washington State University
Spokane, Washington

Alvin H. Schmaier, M.D.

University of Michigan Medical Center
Ann Arbor, Michigan

J.N. Shanberge, M.D.

William Beaumont Hospital
Royal Oak, Michigan

David E. Nevalainen, Ph.D.
Board Liaison

Abbott Laboratories
Abbott Park, Illinois

Julie A. Alexander, M.T.(ASCP), M.A.
Staff Liaison

NCCLS
Wayne, Pennsylvania

Foreword

Since its original description by Quick¹ 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 severe, acute, or chronic hepatic disease. The PT is also the most commonly used test for monitoring oral anticoagulant therapy.

Thromboplastin, a phospholipid/tissue factor preparation, the principal reagent used in the PT, is commercially available in a variety of preparations of primarily animal origin. There are differences among commercial thromboplastin preparations in their sensitivity to reductions in coagulation factors that may affect their usefulness, particularly in the monitoring of oral anticoagulant therapy.²⁻⁵

The activated partial thromboplastin time (APTT) is sensitive to quantitative and qualitative abnormalities in the intrinsic and common pathways of coagulation. It is the most common coagulation procedure performed in routine laboratories, apart from the prothrombin time. The APTT is particularly sensitive to defects of the intrinsic coagulation pathway (Factors VIII, IX, XI, XII, prekallikrein, and high molecular weight kininogen).⁶ It is commonly used for monitoring heparin anticoagulant therapy. It detects other inhibitors of blood coagulation, the most common of which is the lupus anticoagulant, 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 (e.g., 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 sensitivity 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 lupus anticoagulants has been reported.¹¹ Likewise, marked APTT variability in responsiveness to heparin has been observed among commercially available APTT reagents.¹²⁻¹⁴

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.

H47-A 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 pre-analytical and analytical variables, adherence to the recommended techniques will improve precision and accuracy among laboratories. Recommendations on result reporting and safety precautions are provided.

Key Words

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

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

1 Introduction

The results of the prothrombin time (PT) test and activated partial thromboplastin time (APTT) test can be affected by a number of pre-analytical variables, such as method of blood collection, surface characteristics of collection containers, type of anticoagulant, specimen and sample storage conditions, and analytical 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 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.

2 Scope

This document gives general guidelines for performing the PT and APTT by a conventional routine method using citrated, platelet-poor plasma. H47-A does not deal with alternative methods using citrated whole blood, capillary blood obtained by the finger-stick method, or nonclot-based end point detection, such as chromogenic substrate assay.

3 Safety

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with universal precautions. Guidelines for specimen handling are available from the U. S. Centers for Disease Control and Prevention [*MMWR* 1987;36(suppl 2S): 2S–18S]. NCCLS document [M29-T2](#), *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline*, deals specifically with this issue.

4 Glossary of Terms

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

Coagulation Factors: The various substances in plasma involved in the clotting process. The following factors have been identified (synonyms that are or have been in use are included):

- Factor I (fibrinogen)
- Factor II (prothrombin)
- Factor III (thromboplastin, tissue factor)
- Factor IV (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).

Contact activators: Substances that activate the coagulation Factor XII to active proteolytic enzyme. These activators are normally negatively charged particulate substances but may be soluble compounds.

Control (plasma): Used to monitor the stability of the laboratory test system, such as reagents, instruments, reconstituting and diluting fluids, and pipets. It may be in a frozen or lyophilized state. "Normal controls" give test results within range of the reference interval. Abnormal control plasmas should yield abnormally long PTs and APTTs, preferably within the recommended therapeutic range.

Heparin: Standard heparin is a mixture of complex mucopolysaccharides of widely varying molecular weight (5 to 50,000). This is in contrast to low molecular weight heparin and heparinoids.

International normalized ratio (INR): The international normalized ratio, or INR, is the PT ratio obtained by using the appropriate World Health Organization (WHO) international reference preparation as the source of thromboplastin in the performance of a PT. The PT ratio determined by the use of another thromboplastin may be converted to the INR by using the equation: $INR = R^{ISI}$, where R is the PT ratio obtained with the working thromboplastin. The ISI should be determined by standard protocols according to WHO guidelines and provided by the manufacturer to the user for a particular reagent/instrument combination.

International sensitivity index (ISI): The comparative slope, used to calculate the INR, is referred to by the WHO as the international sensitivity index, or ISI.^{15,16} (See Appendix A for further explanation of the ISI.)

Lupus anticoagulant: An immunoglobulin (IgG, IgM, or both) observed in the plasma of patients with systemic lupus erythematosus and a variety of other conditions that interferes with in vitro phospholipid-dependent tests of coagulation (e.g., APTT, dilute Russell's Viper Venom Test). Usually, presence of the lupus anticoagulant is associated with an increased risk of venous and arterial thrombosis.

Partial thromboplastin. A phospholipid preparation that facilitates the in vitro interaction of coagulation factors.

Prothrombin time ratio (PT ratio). The PT ratio is the PT of a test plasma divided by the geo-

metric mean of the reference range. (See Appendix B.)

Sample (patient). Prepared from the patient specimen and used to obtain information by means of a specific laboratory test.

Specimen (patient). A volume of whole blood appropriately collected, transported, and processed to provide a sample for performing one or more laboratory tests.

Thromboplastin. A phospholipid/tissue factor preparation used in the PT that binds Factor VII and VIIa. It facilitates activation of Factor VII to Factor VIIa. The complex of tissue factor and Factor VIIa can activate both Factor IX and Factor X in the presence of calcium ions.

5 Equipment

5.1 Containers

Perform the tests using nonwetable containers.

5.2 Delivery Systems

Use delivery systems supplied with an instrument system with that system. Generic delivery systems may be used. The user should demonstrate and document accurate calibration of all delivery systems used.

5.3 Heating Block

A heating block or waterbath should be available to preheat and/or maintain plasma and reagents at 37 ± 1 °C. If a heating block is used, measurements should be made to ensure that samples are warmed adequately before testing.

6 Specimen Collection, Transport, and Sample Storage

Collect, transport, and store blood according to NCCLS document H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline*.

7 Considerations in Performing the PT and APTT Tests

7.1 Manufacturers' Instructions

Follow the manufacturers' instructions for reagents and equipment.

7.2 Acceptable Variability

Analytical error (see Section 10.4) is influenced by the reagents, instruments, sample delivery devices, and timer, resulting in imprecision. The total day-to-day coefficient of variation (CV) of the analytic system should be less than 5% with the same lot of normal and abnormal control plasmas.

7.3 Reagent Grade Water

Use Type I reagent grade water, as specified in NCCLS document [C3-A2, *Preparation and Testing of Reagent Water in the Clinical Laboratory—Second Edition; Approved Guide-line*](#), or as otherwise specified by the manufacturer. If the laboratory uses a different type of water, it should document its acceptability.

7.4 Calcium Ion Concentration

Use the concentration of calcium ions recommended by the manufacturer of the PT and APTT reagents.

7.5 Conditions of the Test System

Use only clean collection tubes, storage tubes, plastic ware, and delivery systems in the performance of the tests.

7.6 Controls Outside of Stated Limits

If the test values for the control samples are not within the stated limits, check reagents, control plasma, and equipment. Document the identifiable causes and actions undertaken to identify and correct the problem before any patient plasma data are reported.

7.7 Control Plasma Collection, Handling, and Storage

If control plasma samples are prepared within the laboratory, they must be prepared and stored

according to acceptable methods. Collect blood used for preparation of control plasmas into citrate anticoagulant. The citrate solution and ratio of citrate to blood volume should be identical to that used in the collection of test specimens. Handle and store control plasma(s) under conditions identical to, or as similar as possible to, those used for storage of test samples. See NCCLS document [H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline*](#) for more information on coagulation specimen collection, handling, and storage.

7.8 Frequency of Control Testing

Test controls at the initiation of testing each day and at least once each shift, or with each group of assays. Controls should also be tested with each reagent change or major instrument adjustment. In laboratories where there is a heavy workload of PTs and/or APTTs, test a normal and an abnormal control at a minimum of every 40 samples.

7.9 Reproducibility of Duplicates

The size of the difference between duplicate measurements is commonly used as a criterion for result acceptability. This is helpful as a check on system imprecision and/or sporadic analytic errors. Although the exact size of difference that constitutes the appropriate operational limit may vary with the analytic system used, the difference between duplicate results should agree within 10% of their mean value.

7.10 Reference Intervals

A reference interval should be established by each laboratory and it should be verified with any change in reagent lot number, instrument, collection system, or at least once a year. For more information on reference intervals, see NCCLS document [C28-A, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory; Approved Guideline*](#).

7.11 General Quality Control

The laboratory should follow generally accepted quality control practices. Specifically, laboratory personnel with appropriate experience should inspect the quality control results daily to evaluate for trends or shifts, as well as out-of-limit results. Individual patient values should be reviewed to look for unusual or unlikely patterns that can indicate a system malfunction or clerical errors. Maintenance of all instruments should be carried out in accordance with manufacturers' directions and all actions documented. In addition, there should be periodic review (generally monthly) of quality control data to look for long-term changes in the analytic systems and, when appropriate, for the comparison of results with those of a peer group.

Each laboratory should enroll in a proficiency testing program acceptable to the relevant inspecting and accrediting agencies.

The laboratory should keep accurate and complete records of the lot numbers of reagents, reference materials, and, where possible, evacuated tubes (if used).

8 Performance of the Prothrombin Time (PT) Test

8.1 PT Principle

Thromboplastin and a source of calcium ions are combined with test plasma at 37 °C; the PT is the time, in seconds, required for a detectable fibrin clot to form.

8.2 PT Reagents: Thromboplastins

Usually, the thromboplastin reagent is a buffered thromboplastin–calcium mixture supplied by the manufacturer. There is great variability in ISI values in the responsiveness of different PT reagents.

8.3 PT Performance Temperature

Perform the test at 37 ± 1 °C. Prewarm aliquots of plasma to 37 °C for no more than 10 minutes before performing the test. Follow the manufacturers' instructions that describe preparation and handling of individual thromboplastin reagent.

8.4 PT Test Procedure

Initiate the PT by mixing two parts of prewarmed thromboplastin–calcium reagent and one part of prewarmed citrated plasma. Start a timing device the instant the reagents are mixed. Record the time required for clot formation.

8.5 PT End Point

Measure the end point by a variety of optical or electromechanical methods using manual, semi-automated, or automated devices. Determinations are commonly performed in duplicate and the mean of the two values is reported. With improvements in the precision of semi-automated and automated coagulation instruments, singlet testing is acceptable, if appropriate quality standards are met.¹⁷ For more information on single versus duplicate determinations, refer to NCCLS document H21-A2.

8.6 Reporting PT Results

The laboratory should report the results of the PT test to the nearest half of a second or less along with the normal reference interval and the INR. The ratio of the PT to the geometric mean of the reference interval may also be reported. It has been shown that for patients stabilized on oral anticoagulant therapy, the reporting of an INR is preferred because it reduces intermethod variability.^{2,4,5,15,16,18} Conversion to an INR in effect calibrates the results of a particular reagent/instrument system to results of an international reference reagent. Despite this, INR values produced by different test systems can still vary considerably.¹⁹ This variability is diminished by universal use of highly responsive thromboplastin reagents, with an ISI below 1.5.

9 Performance of the APTT Test

9.1 APTT Principle

Citrated test plasma, a contact activator, and procoagulant phospholipids are mixed and incubated at 37 °C. The contact agent acti-

vates Factor XI and Factor XII. The phospholipid provides a surface for interaction of coagulation factors. After incubation, an appropriate concentration of calcium ions is added, and time to clot formation is measured. Calcium ions promote activation of the intrinsic coagulation cascade subsequent to Factor XIa.

9.2 APTT Reagent

The APTT reagent is a mixture of partial thromboplastin and contact factor activator. The activator may be celite, kaolin, silica, ellagic acid or other suitable substances. The APTT reagent/instrument combination should be able to detect abnormally prolonged results with plasmas that have less than 0.3 U/mL (30% factor activity) of the following coagulation factors: VIII, IX, XI.

9.3 APTT Performance Temperature

Perform the test at 37 ± 1 °C.

Prewarm aliquots of plasma at 37 °C for no more than 10 minutes before performing the test. Follow the manufacturers' instructions that describe preparation and handling of individual APTT reagents.

9.4 Contact Activation Time

The contact activation time refers to the duration of incubation of test plasma and APTT reagent. Rigid standardization of contact activation time is important. Because this varies with the instrument and particular APTT reagent used, follow the manufacturer's instructions. For manual procedures, use a stopwatch or a similarly accurate timing device.

9.5 APTT Test Procedure

The APTT is a two-stage test. Initiate the first stage by mixing one part APTT reagent (see [Section 9.2](#)) and one part citrated plasma. Simultaneous with the mixing of the reagent and the plasma, start a timing device to measure the exact contact activation time. At the end of the recommended activation time, initiate the second stage by adding one part of

prewarmed calcium chloride (see [Section 7.4](#)), and simultaneously starting a timer. Record the time required for clot formation.

9.6 APTT End Point

The end point is the formation of a fibrin clot. It can be measured by a variety of optical or electromechanical methods using manual, semi-automated, or automated devices. Determinations are commonly performed in duplicate and the mean of the two values is reported. With improvements in the precision of semi-automated and automated coagulation instruments, singlet testing is acceptable, if appropriate quality standards are met.¹⁷ For more information on single versus duplicate determinations, refer to NCCLS document [H21-A2](#).

9.7 Heparin Sensitivity²⁰

Because the APTT is commonly used for monitoring heparin therapy, the APTT reagent/instrument system should be adequately responsive to standard heparin. The therapeutic APTT range for heparin therapy should be determined in each hospital laboratory by establishing the APTT range corresponding to a recommended heparin concentration range, preferably using plasma from patients on heparin therapy (*ex vivo*). This requires the availability of an assay for measuring heparin concentration (e.g., protamine sulfate titration, anti Xa chromogenic assay). The therapeutic APTT range determined using plasmas from normal subjects spiked *in vitro* with known heparin concentrations is higher generally, but it is also acceptable.

9.8 Lupus Anticoagulants²¹

APTT reagents are variably sensitive or insensitive to lupus anticoagulants. The reagent manufacturer should provide sufficient documentation with respect to lupus anticoagulant sensitivity. Also, national proficiency testing programs provide appropriate additional information about differences in reagent sensitivity to lupus anticoagulants. Note, however, that there is considerable heterogeneity among individual pa-

tients; consequently, no single reagent will detect all lupus anticoagulants.

9.9 Reporting of APTT Results

The laboratory should report the results of the APTT test to the nearest second or less along with the reference interval.

10 Sources of Error

10.1 Specimen- or Sample-Related Problems

Following are several specimen- or sample-related problems:

- Overfill or underfill of collection tubes
- Failure to correct the citrate volume for persons with high (>0.55) packed cell volume (PCV; hematocrit) (See NCCLS document [H21-A2, Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline](#))
- Incorrect volume, type (e.g., EDTA or oxalate), or concentration of anticoagulant in the collection tube
- Clotted, hemolyzed, icteric, or lipemic specimens (refer to NCCLS document [H21-A2, Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline](#), for more information)
- Inadequate or too vigorous mixing of the specimen with the reagents
- Contaminated collection or storage tubes
- Contamination with heparin
- Improper or defective specimen collection tubes.

10.2 Reagent-Related Problems

Following are several reagent-related problems:

- Contaminated reagents
- Reconstitution with incorrect diluent volume
- Reconstitution with other than the recommended diluent
- Defects in the reagent due to mishandling in shipping or storage
- Use of the reagent beyond the stated reconstituted stability date or beyond the expiration date.

10.3 Other Pre-Analytical Errors

Pre-analytical errors include delay in or use of nonstandardized procedures for transporting, processing, storing, or testing the specimen.¹⁶ (See NCCLS document [H21-A2, Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline](#), for more information.)

10.4 Analytical Errors

Analytical errors (see [Section 7.2](#)) can be due to the following circumstances:

- Incorrect incubation time or activation time
- Inaccurate or imprecise dispensing of reagents
- Failure to use proper instrument operating procedures
- Instrument malfunction, such as defective bulb, incorrect temperature, reagent splash, poor reagent delivery, or electrical interferences.

References

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Appendix A: Explanation of the International Sensitivity Index (ISI)

The International Sensitivity Index (ISI):

The ISI is a mathematical indicator of the responsiveness of a PT testing system to deficiencies of the Vitamin K coagulation factors. *A low ISI indicates a highly responsive PT system and a high ISI indicates a poorly responsive system. The WHO reference thromboplastin is highly responsive and has an assigned ISI value of 1.0.* Usually, the ISI for a specific test system is determined by the thromboplastin manufacturer and is used to convert an observed PT Ratio to its INR equivalent by means of the equation:

$$INR = (PT\ Ratio)^{ISI}.$$

Taking the logarithms of both sides of the equation, we have:

$$\log(INR) = ISI - \log(PT\ Ratio).$$

The equation above is that of a straight line with a slope of *ISI* and an intercept of 0. In other words, the ISI is the slope of the line that defines the relationship between INRs and the observed PT Ratios. The line defined by the equation is the regression line (i.e., the line of best fit) determined by plotting the logarithms of the reference INR values against the logarithms of the PT Ratios of the test system. The regression line is derived from the logarithms of the values and not the values themselves because this transformation was determined to better conform to the assumptions implicit in regression analysis. It can be shown that the ISI is also the slope of the regression line of logarithms of the INRs plotted against the logarithms of the actual PTs (not the ratios). In this case, the intercept of the regression line is not zero, however.

Appendix B: Description of the Geometric Mean (GM)

The geometric mean is calculated by the following equation:

$$GM = \sqrt[n]{X_1 \cdot X_2 \cdot X_3 \dots \cdot X_n}$$

Taking the logarithm of both sides, we have:

$$\log(GM) = [\log(X_1) + \log(X_2) + \log(X_3) + \dots + \log(X_n)]/n.$$

Taking the antilog of both sides, yields:

$$GM = \text{antilog}\{[\log(X_1) + \log(X_2) + \log(X_3) + \dots + \log(X_n)]/n\}.$$

The GM, therefore, is the antilog of the arithmetic mean of the logarithms of the individual values of interest. When the distribution of values is distributed normally, the GM, the arithmetic mean, the median, and the mode of the population being studied are identical theoretically. These values diverge from each other, however, as the population distribution becomes more skewed. The GM, is a more appropriate estimate of the average value than the arithmetic mean when the population of interest is lognormally* distributed because the GM takes skewing into account. In this situation, the GM and the median remain identical theoretically. Because the PTs of the healthy population are thought to be lognormally distributed, the International Committee on Thrombosis and Hemostasis recommends use of the GM as the denominator in calculating PT ratios in order to maintain mathematical correctness.

*

Lognormal distributions are distributions where the logarithms of the individual values (but not necessarily the values themselves) are normal, i.e. Gaussian, in distribution. They are frequently encountered in nature in a variety of situations.

Summary of Comments and Subcommittee Responses

H28-T: *One-stage Prothrombin Time Test (PT); Tentative Guideline*

General

1. Perhaps the standards should heavily emphasize the use of appropriate reagents: that is, use of sensitive reagents that provide extended therapeutic ranges. A comparison of the original results published by Dr. Quick with those of the widely used reagents today will show the drop in reagent quality.
 - **The subcommittee included an expanded explanation of the international sensitivity index (ISI) in Appendix A of H47-A.**
2. The abandonment of the pH recommendation is of concern. There must be some consensus on the optimum pH that can be included in the document.
 - **In the subcommittee's experience, the pH requirements are dependent on the composition of the reagents; therefore, a pH recommendation is not provided.**

Section 4

3. Prothrombin time ratio (PT ratio) "...divided by the median of the reference range." The word "median" should be replaced by "mean."
 - **The subcommittee agrees and the text is revised to read "geometric mean."**

Section 6.1

4. In Section 6.1 (now Section 5.1), a recommendation to use nonwetable containers is stated. Then, in the answer to Comment #17 in the comment summary section, a statement is made that if a specimen is collected in a wettable surface tube, it must be transferred to a nonwetable surface as soon as possible. Please provide clear directions and recommendations.
 - **The recommendation is clarified; see Section 5.1 of H47-A.**

Section 8.3 (now Section 7.2)

5. I suggest that terms like "5% (total analytical variability)" be clarified by including the confidence limit.
 - **The subcommittee believes that the coefficient of variation (CV) is more appropriate and that it is an expression of laboratory day-to-day precision.**

Section 8.4 (now Section 7.3)

6. Only specified conditions require the use of Type I reagent grade water. This ionically pure water (highest grade) is recommended when a minimum level of ionized constituents is important. Our high volume laboratory assays 350 specimens/day using coagulation reagents with preservatives. Using Type I water is not necessary.

Summary of Comments and Subcommittee Responses (Continued)

- **The document is revised in response to this comment (see Section 7.3 of H47-A). The subcommittee recommends the use of Type I reagent water because it is considered to be the "ideal" water that can be produced with currently available water treatment/purification technology, but other types of water may also be appropriate and, as such, the recommendations of the manufacturer may be followed. If the laboratory uses a different type of water, it should document its acceptability.**

Section 8.9 (now Section 7.11)

7. The proposed stipulation of recording lot numbers of the specimen tubes poses problems for this laboratory and (I suspect) for many other large hospital and independent laboratories. To record lot numbers on specimens received from wards, intensive care units, emergency rooms, and out-patient clinics in an 1100-bed hospital—as well as from more than 500 private physician's offices—is unrealistic. Furthermore, such a requirement would only increase dwell time and compromise patient care.
- **The recommendation is for the laboratory or patient care unit to keep records of tube lot numbers used (in bulk) by the facility, where possible, but not the lot numbers of each individual tube used for each patient. In the past, there have been documented occurrences of error affecting patient care because of defective tubes. For this reason, the subcommittee believes the recommendation is appropriate (References: Heynes AD, VanBerg DJ, Kleynhans PH, DuToit PW. Unsuitability of evacuated tubes for monitoring heparin therapy by the activated thromboplastin time test. *J Clin Path* 1981;34(1):63–68 and Palmer RN, Gralnik HR. Inhibition of the cold activation of Factor XI and the PT time. *Am Clin Pathol* 1984;81(5): 618–622.)**

Section 8.12 (now Section 7.8)

8. The document appears to indicate use of a normal and abnormal control. It has been common laboratory practice to utilize three controls in coagulation. Are we interpreting this correctly to conclude that two controls are now deemed adequate?
- **The subcommittee no longer believes that three controls are obligatory.**
9. With our volume of 350 specimens a day, testing an abnormal control with every 40 specimens would be counterproductive. In addition to the low, normal, and high controls run routinely every shift, we re-run a normal control with every 20 samples throughout the 24 hours. Furthermore, because we do not perform a linearity study to establish the upper limit of the curve, testing a normal control should be indicative of any reagent deterioration.
- **The subcommittee recommends testing an abnormal control because it is more sensitive to detecting early changes in the system.**
10. Does a "group" of assays include groups of one (i.e., stat tests)? Please clarify.
- **The subcommittee considers a group of one as a group.**

Summary of Comments and Subcommittee Responses (Continued)Section 8.15 (now Section 7.10)

11. This document indicates that a new reference interval should be calculated each time we change reagent lots or lot numbers of evacuated blood collection tubes. We are concerned with the feasibility and the effort involved in confirming reference intervals for each change in blood collection tubes. Have studies been conducted to indicate the frequency of problems associated with changes in lot numbers of blood collection systems? Can you please provide us with any such studies or further references on this topic?
- **Section 7.10 has been revised to state that the reference interval should be verified with any change in the collection system (e.g., a change from glass tubes to plastic tubes), not a change in the lot number of the tubes.**
12. "The test results should be analyzed statistically and a ± 2 SD or 95% confidence limit calculated." The word "mean" should be inserted before " ± 2 SD."
- **The text has been revised to refer the reader to NCCLS document C28-A, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory; Approved Guideline*, for information on definitions and reference intervals.**
13. If a reasonably large batch of normal plasma is used, the results will show a significant "tail" to the right. "Mean and 2SD" is not an appropriate choice for determining the interval, because it will lead to an inappropriate range.
- **The subcommittee believes that the mean ± 2 SD is a robust estimate of population distribution, indicating a 95% confidence limit. By defining the normal range in this manner, patients with abnormal results will be less likely to go undetected. It is recommended (in Section 7.11) to refer to NCCLS document C28-A, *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline*, for information on defining reference intervals.**

Section 9 (now Section 8.6)

14. "...the reference interval and the median of that interval..." The word "median" should be replaced by "mean."
- **The subcommittee agrees and the text is revised to read "geometric mean."**
15. Section 9 indicates that results can be reported to the nearest half second or the ratio of the PT to the median of the reference intervals. If neither of these seem appropriate, then consider reporting the answer as an international normalized ratio. Surely, by now, one or another of these has advantages.
- **The text is revised to recommend reporting of results of the PT test to the nearest half of a second or less along with the normal reference interval and the international normalized ratio (INR).**
16. More discussion of the international normalized ratio should be included. It appears that the INR is one way of reducing lab/lab variability.

Summary of Comments and Subcommittee Responses (Continued)

- **The document is revised in response to this comment.**
- 17. Please do not encourage the use of ratio of result and mean of reference interval. Enough damage has already been caused by the confusion between a simple arithmetic relationship and the more complicated hyperbolic curve that is reality.
- **The subcommittee believes that it is appropriate to provide this as an option since there is still divergent opinions in the field related to this issue.**

Summary of Comments and Subcommittee Responses

H29-T: *Activated Partial Thromboplastin Time Test (APTT); Tentative Guideline*

Section 8.4 (now Section 7.3)

1. Only specified conditions require the use of Type I reagent grade water. This ionically pure water (highest grade) is recommended when a minimum level of ionized constituents is important. Our high-volume laboratory assays 350 specimens/day using coagulation reagents with preservatives. Using Type I water is not necessary.
- **The document is revised in response to this comment (see Section 7.3 of H47-A). The subcommittee recommends the use of Type I reagent water because it is considered to be the "ideal" water that can be produced with currently available water treatment/purification technology, but other types of water may also be appropriate and, as such, the recommendations of manufacturer may be followed. If the laboratory uses a different type of water, it should document its acceptability.**

Section 8.10 (now Section 7.11)

2. The proposed stipulation of recording lot numbers of the specimen tubes poses problems for this laboratory and (I suspect) for many other large hospital and independent laboratories. To record lot numbers on specimens received from wards, intensive care units, emergency rooms, and out-patient clinics in an 1100-bed hospital—as well as from more than 500 private physician's offices—is unrealistic. Furthermore, such a requirement would only increase dwell time and compromise patient care.
- **The recommendation is for the laboratory or patient care unit to keep records of tube lot numbers used (in bulk) by the facility, where possible, but not the lot numbers of each individual tube used for each patient. In the past, there have been documented occurrences of error affecting patient care because of defective tubes. For this reason, the subcommittee believes the recommendation is appropriate. References: Heynes, AD, VanBerg, DJ, Kleynhans, PH, DuToit, PW, Unsuitability of evacuated tubes for monitoring heparin therapy by the activated thromboplastin time test. *J. Clin Path.*, 1981, Vol. 34(1), pages 63-68 and Palmer, RN, Gralnik, HR, Inhibition of the cold activation of Factor XI and the PT time. *Am. Journal Clin. Path.* 1984 Volume 81(5), Pages 618-622.**

Section 8.13 (now Section 7.8)

3. With our volume of 350 specimens a day, testing an abnormal control with every 40 specimens would be counterproductive. In addition to the low, normal, and high controls run routinely every shift, we re-run a normal control every 20 samples throughout the 24 hours. Furthermore, because we do not perform a linearity study to establish the upper limit of the curve, testing a normal control should be indicative of any reagent deterioration.
- **The subcommittee recommends testing an abnormal control because it is more sensitive to detecting early changes in the system.**
4. The document appears to indicate use of a normal and abnormal control. It has been common laboratory practice to utilize three controls in coagulation. Are we interpreting this correctly to conclude that two controls are now deemed adequate?

Summary of Comments and Subcommittee Responses (Continued)

- **The subcommittee no longer believes that three controls are obligatory.**

Section 8.18 (now Section 7.10)

5. This document indicates that a new reference interval should be calculated each time we change reagent lots or lot numbers of evacuated blood collection tubes. We are concerned with the feasibility and the effort involved in confirming reference intervals for each change in blood collection tubes. Have studies been conducted to indicate the frequency of problems associated with changes in lot numbers of blood collection systems? Can you please provide us with any such studies or further references on this topic?
- **Section 7.10 has been revised to state that the reference interval should be verified with any change in the collection system (e.g., a change from glass tubes to plastic tubes), not a change in the lot number of the tubes.**

Related NCCLS Publications

- C3-A2** **Preparation and Testing of Reagent Water in the Clinical Laboratory—Second Edition; Approved Guideline (1991).** C3-A2 addresses the proper water requirements for Class I, II, and III water. It also describes appropriate quality control procedures and testing for microbiological content.
- C28-A** **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (1992).** C28-A addresses a protocol for the determination of reference ranges for defined populations as an aid to the interpretation of laboratory data.
- H1-A3** **Evacuated Tubes for Blood Specimen Collection—Third Edition; Approved Standard (1991).** H1-A3 addresses requirements for blood collection tubes and additives.
- H3-A3** **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture—Third Edition; Approved Standard (1991).** H3-A3 discusses methods for the collection of blood specimens by venipuncture, including a description of an appropriate training program aimed at increasing analyte integrity and minimizing laboratory error.
- H18-A** **Procedure for the Handling and Processing of Blood Specimens; Approved Guideline (1990).** H18-A addresses the multiple factors affecting the handling and processing of blood specimens that can introduce test result imprecision or systematic bias.
- H21-A2** **Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline (1991).** H21-A2 addresses procedures for collecting, transporting, and storing blood samples, preparing plasma for coagulation testing, and for performing the tests.
- H30-A** **Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline (1994).** H30-A gives general guidelines for performing the fibrinogen assay in the clinical laboratory. It includes a discussion of interpretation of results and in vivo and in vitro conditions that can alter results.
- H34-P** **Determination of Factor VIII Coagulant Activity (VIII:C); Proposed Guideline (1986).** H34-P discusses a method for determining Factor VIII coagulant activity, specimen collection and handling, reagents, materials, sources of error, and interpretation of the test.
- H40-P** **Determination of Factor IX Coagulant Activity; Proposed Guideline (1986).** H40-P discusses a method for determining Factor IX coagulant activity; specimen collection, transport, and sample storage; reagents and materials; and interpretation of test results.
- M29-T2** **Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline (1991).** M29-T2 gives guidance on the risk of transmission of hepatitis B virus and the human immunodeficiency virus in the laboratory. It describes specific precautions for preventing the transmission of bloodborne infection during clinical and anatomical laboratory procedures.