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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.



Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

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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 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 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.