



# H47

## One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test

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

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## Abstract

Clinical and Laboratory Standards Institute document H47—*One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test* 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 vitamin K antagonist therapy. The APTT measures the intrinsic coagulation pathway and is used in monitoring heparin therapy, as well as parenteral direct thrombin inhibitor anticoagulant therapy such as argatroban and lepirudin. 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.

Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test*. 3rd ed. CLSI guideline H47 (ISBN 978-1-68440-172-7 [Print]; ISBN 978-1-68440-173-4 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2023.

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## Suggested Citation

CLSI. *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test*. 3rd ed. CLSI guideline H47. Clinical and Laboratory Standards Institute; 2023.

### Previous Editions:

June 1996, May 2008

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H47-Ed3

ISBN 978-1-68440-172-7 (Print)

ISBN 978-1-68440-173-4 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 43, Number 5

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## Foreword

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Since its original description by Quick<sup>1</sup> 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, as well as vitamin K deficiency or disseminated intravascular coagulation. The PT is also the most commonly used test for monitoring vitamin K antagonist (VKA) therapy. The PT may also be prolonged by direct oral anticoagulant (DOAC) therapy, depending on the nature and concentration of the DOAC used.

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 VKA therapy.<sup>2-6</sup>

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).<sup>7,8</sup> 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 are sufficiently decreased to cause clinical bleeding (<30%).<sup>9</sup> 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.<sup>7,8,10,11</sup> A similarly variable sensitivity of the APTT to circulating LAs has been reported.<sup>12</sup> Likewise, marked APTT variability in responsiveness to heparin and DOAC therapy has been observed among commercially available APTT reagents.<sup>8,13,14</sup>

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<sup>15</sup> and H57.<sup>16</sup>

## Overview of Changes

This guideline was revised in 2023 under the Limited Revision Process and replaces the second edition of the guideline, which was published in 2008. Several changes were made in this edition, including:

- Adding new anticoagulants
- Adding effect of hemophilia therapies on APTT
- Adding new references

**NOTE:** The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

### KEY WORDS

activated partial  
thromboplastin time (APTT)

citrate

coagulation

coagulation factor(s)

control (plasma)

fibrinogen

international normalized ratio  
(INR)

international sensitivity index  
(ISI)

phospholipids

prothrombin time (PT)

thrombin time

thromboplastin

tissue factor

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# One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test

## 1 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,<sup>17</sup> 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.

### 1.1 Scope

H47 provides general guidelines for performing PT and APTT tests with the conventional method of using citrated, platelet-poor plasma. It does not cover alternative methods of using citrated whole blood or capillary blood obtained by the fingerstick method. Nonclotting-based, end-point-detection tests, such as chromogenic substrate assays, are also not discussed.

### 1.2 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 US Centers for Disease Control and Prevention.<sup>18</sup> 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.<sup>19</sup>

## 4 Considerations in Performing the PT and APTT Tests

### 4.1 Manufacturer's Instructions

Follow the manufacturer's instructions for reagents and equipment. Also see CLSI document H57<sup>16</sup> for evaluating coagulometers.

### 4.2 Acceptable Variability

Analytical (examination) error 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 analytical system should be less than 5% with the same lot of normal and abnormal control plasmas.

### 4.3 Reagent Grade Water

Use the grade of water specified by the manufacturer. If the manufacturer does not specify, then use clinical laboratory reagent water (CLRW), as specified in CLSI document GP40.<sup>26</sup> If the laboratory uses a different type of water than specified by the manufacturer or CLRW, it should document its acceptability.

### 4.4 Calcium Ion Concentration

Use the concentration of calcium ions recommended by the manufacturer of the specific PT and APTT reagents in use in the laboratory.

### 4.5 Conditions of the Test System

Use only clean collection tubes, storage tubes, plasticware, and delivery systems in the performance of the tests. All surfaces should not interact with the sample or any reagents.

### 4.6 Controls Outside Stated Limits

If the test values for the control samples are not within the stated limits, check the reagents, control plasma, and equipment. Document the identifiable causes and actions undertaken to identify and correct the problem before any patient data are reported. For additional details on evaluating control performance, refer to CLSI document C24.<sup>27</sup>

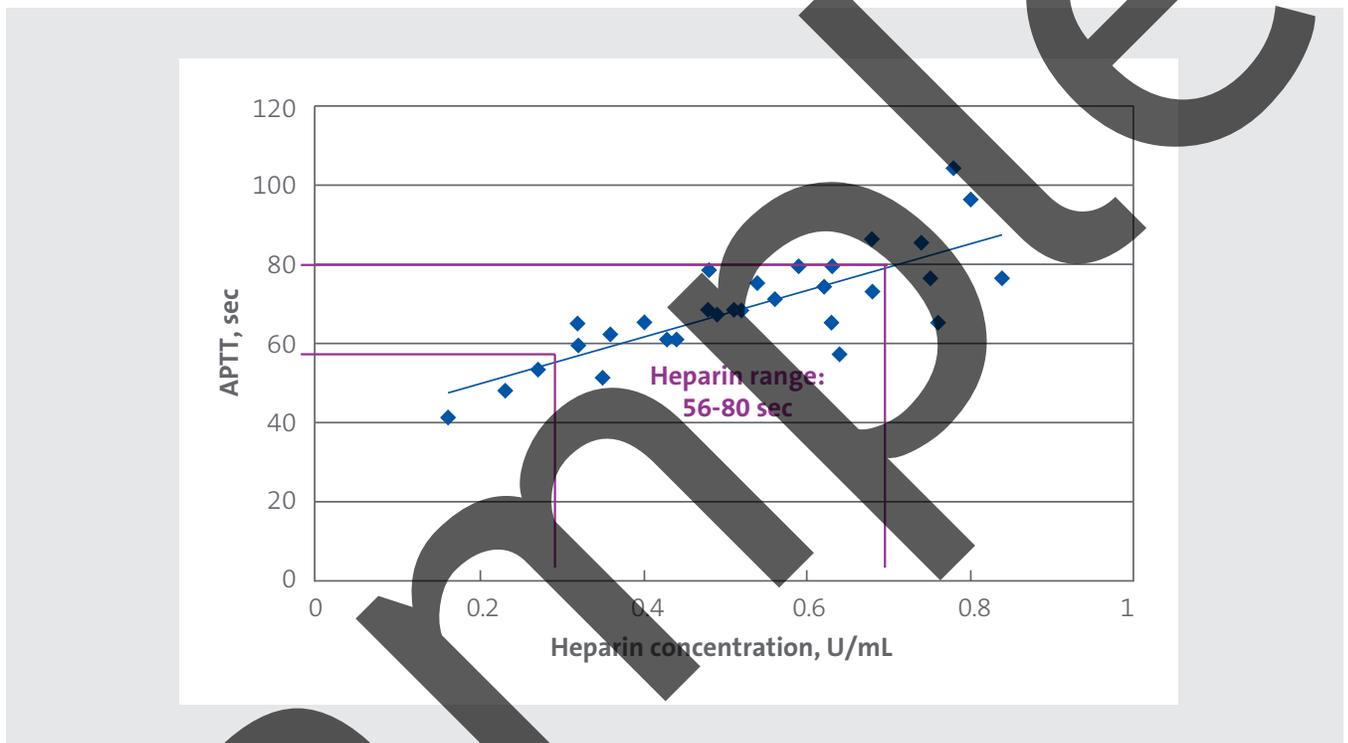
### 4.7 Control Plasma Collection, Handling, and Storage

Commercially available control plasmas are recommended, if available to the laboratory. If control plasma samples are prepared within the laboratory, they must be prepared and stored according to acceptable methods (see CLSI document EP23<sup>28</sup>). 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. If relevant, handle and store control plasma(s) under conditions identical to, or as similar as possible to, those used for storage of test samples. See CLSI document H21<sup>17</sup> for more information on coagulation specimen collection, handling, and storage.

## Appendix C. (Continued)

3. To determine the acceptability of the new reagent-instrument system, perform a cumulative summation of differences.<sup>1</sup>
  - a. Sum the data for the old and new reagent-instrument systems.
  - b. Determine the mean and SD for each set of data points.
  - c. If the difference is greater than five seconds, then there is concern that the reagents are not the same.
  - d. This can be continued for a cumulative evaluation of successive lots. The cumulative change of multiple lots and/or years should never exceed five seconds.



Abbreviations: APTT, activated partial thromboplastin time; sec, second(s); UF, unfractionated.

**Figure C1. Example of an Ex Vivo UF Heparin Therapeutic Range Determination**

### References for Appendix C

- 1 Olson JD, Arkin CF, Brandt JT, et al. College of American Pathologists Conference XXXI on laboratory monitoring of anticoagulant therapy: laboratory monitoring of unfractionated heparin therapy. *Arch Path Lab Med.* 1998;122:782-798.
- 2 Brill-Edwards P, Ginsberg JS, Johnston M, Hirsch J. Establishing a therapeutic range for heparin therapy. *Ann Intern Med.* 1993;119:104-109.