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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.]

THE NCCLS consensus process, which is the mechanism for moving a document through two or more levels of review by the clinical laboratory testing community, is an ongoing process. (See the inside front cover of this document for more information on the consensus process.) Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, bench and reference methods, and evaluation protocols used in clinical laboratory testing, users should replace outdated editions with the current editions of NCCLS documents. Current editions are listed in the *NCCLS Catalog*, which is distributed to member organizations, or to nonmembers on request. If your organization is not a member and would like to become one, or to request a copy of the *NCCLS Catalog*, contact the NCCLS Executive Offices. Telephone: 610.688.0100; Fax: 610.688.0700.

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Contents

Contents (Continued)

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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 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), anti-hemophilic 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.