



# H48

## Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay

This guideline provides recommendations regarding the proper collection and handling of specimens, reagents, controls, calibrators, and materials needed to optimize factor assay testing. It includes recommendations for good laboratory practices related to analyzer and reagent performance, reference intervals, lot-to-lot validation, and quality control. Assay limitations and sources of errors and variability are also included.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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## Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay

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### Abstract

Clinical and Laboratory Standards Institute guideline H48—*Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay* provides information to be used in harmonizing laboratory testing of factor assays. It provides laboratories with guidelines to optimize factor assay testing by minimizing the effect of variation in preexamination, examination, and postexamination processes. It identifies good laboratory practices related to analyzer and reagent performance, reference intervals, lot-to-lot validation, quality assurance, and quality control issues. Standardizing assay performance provides patients with the best outcomes with regard to both diagnosis and treatment. This guideline is written for laboratorians and/or diagnostic testing personnel responsible for factor assay testing, physicians (eg, hematologists, pathologists) responsible for interpreting results, external quality assessment programs, and manufacturers of factor assay testing reagents and test systems.

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## Foreword

Quantitative assays for measuring coagulant activity of both the intrinsic and extrinsic coagulation factors are important laboratory tools. The factor assay provides valuable information in:

- Patients found to have a prolonged activated partial thromboplastin time (APTT) or prothrombin time (PT)
- Patients with normal coagulation screening test values but a clinically suspected bleeding disorder
- Monitoring factor replacement therapy
- Risk assessment of premature atherosclerotic vascular disease in which elevated activity of Factors VII and VIII have been demonstrated

In addition, factor activity determinations are needed to evaluate the potency of therapeutic factor preparations such as fresh frozen plasma and factor concentrates.

This guideline provides recommendations for the routine performance of one-stage coagulation factor assays that are based upon the conventional APTT and PT coagulation tests described in CLSI document H47.<sup>1</sup> Recommendations on result reporting and safety precautions are also presented.

## Overview of Changes

This guideline replaces the previous edition of the approved guideline, H48-A, published in 1997. Several changes were made in this edition including:

- Expanded terminology
- Use of factor assays to aid in diagnosis of coagulation disorders
- Enhanced preexamination, examination, and postexamination activities and sources of error
- Identification and reporting of inhibitors
- Anticoagulation effect on factor assays
- Reagents and reagent responsiveness
- Lot-to-lot verification

**NOTE:** The findings and conclusions in this guideline are those of the authors and are supported by the CLSI consensus process, and do not necessarily reflect the views of the organizations the authors represent.

## Key Words

Activated partial thromboplastin time, calibration, coagulation factor, extrinsic factor pathway, factor activity, factor assay curve, intrinsic factor pathway, inhibitor, prothrombin time

# Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay

## Chapter 1: Introduction

This chapter includes:

- Guideline scope and applicable exclusions
- Background information pertinent to the guideline content
- Standard precautions information
- “Note on Terminology” that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the guideline
- Abbreviations and acronyms used in the guideline

Historically, testing of blood plasma factors and platelets depended on seeing the clotting process directly or microscopically. Instrumentation later provided mechanical registration of clot development that allowed more reproducible timing and an expression of the clotting process.<sup>2,3</sup>

### 1.1 Scope

This guideline provides specifications for the one-stage clotting factor assay. It is intended to increase the diagnostic usefulness of the one-stage factor assay by providing the laboratory with necessary tools to minimize the effects of variables and to provide guidelines to enhance the precision and accuracy of patient results. Preexamination, examination, and postexamination issues specific to factor activity testing are covered.

This guideline is written for laboratory and/or diagnostic testing personnel responsible for factor assay testing including the performance, QC, and reporting of assays of coagulation factor activity, physicians (eg, hematologists, pathologists) responsible for interpreting results, external quality assessment (EQA) programs, and manufacturers of factor assay testing reagents and test systems.

This guideline does not cover chromogenic, two-stage clotting, antigenic, or manual methodologies for factor assays. Assays for fibrinogen, von Willebrand Factor (VWF), Factor XIII (FXIII), or contact factors of high molecular weight kininogen or prekallikrein are not covered in this guideline. Assays used to quantify inhibitors to specific factors are not covered in this guideline.

### 1.2 Background

The one-stage factor assay is based on the ability of the test plasma to correct the activated partial thromboplastin time (APTT) or prothrombin time (PT) of a specific factor-deficient plasma. The factor activity is quantified with a factor-specific calibration curve prepared using a referenced calibration plasma and a substrate plasma deficient in the factor being tested. Factor assays within the scope of this guideline include Factor II (prothrombin [FII]), Factor V (FV), Factor VII (FVII), Factor VIII (FVIII), Factor IX (FIX), Factor X (FX), Factor XI (FXI), and Factor XII (FXII).

In these assays, either an APTT or a PT is performed on mixtures of diluted test plasma and plasma deficient in the specific coagulation factor being assayed. In general, the APTT test is used for factor activity assays of the intrinsic pathway (Factors VIII, IX, XI, XII, high molecular weight kininogen, and prekallikrein). The PT test is used for those factors of the extrinsic and common pathways (Factors II, V, VII, and X). The observed clotting times are converted into units of factor activity by reading from the time in seconds against a calibration curve constructed from a calibration plasma of known factor activity.

Like the APTT and PT on which they are based, the one-stage assays of coagulation factor activity may be affected by many preexamination and examination variables. In addition, the preparation of the calibration curve and the interpretation of patient values present a considerable potential for test result variation. This has made interlaboratory standardization difficult. The techniques recommended in this guideline are intended to minimize the effects of these sources of error and improve both intra- and interlaboratory precision.

Factor assays quantify the hemostatic capability of a specific soluble procoagulant protein to produce a plasma-based fibrin clot, in a PT- or an APTT-based reaction environment. The specific factor activity of a plasma sample is measured by its ability to correct the prolonged clotting time of substrate plasma deficient in the factor of interest. The measurement of time elapsed between the initiation of the clotting process and clot formation (clotting time) is inversely proportional to the activity of the test factor.

Factor assays are performed more routinely in laboratories today with the advent of available coagulation automation, improved coagulation reagents, and greater standardization of coagulation methods. When a prolongation of the PT and/or APTT occurs, factor assays may be performed as part of the diagnostic algorithm for the patient. Factor assays are commonly used as an aid in:

- Diagnosis of an acquired or inherited factor deficiency of the extrinsic, intrinsic, and/or common pathway
- Determination of the presence of a specific factor inhibitor
- Investigation of the presence of a nonspecific inhibitor (eg, a lupus anticoagulant)
- Determination of the presence of anticoagulant and procoagulant therapy
- Determination of the presence of an acute phase reactant (FVIII) or persistent hereditary evaluation (requiring confirmation by family studies) that may predispose a patient to thrombosis

A factor assay is performed by mixing diluted patient plasma and factor-deficient plasma, and subsequently adding the appropriate base reagent (either PT or APTT reagent). The reagents used in this assay consist of:

- APTT reagent and calcium chloride consisting of a phospholipid and an activator
- Plasma deficient in the factor being tested
- Calibration plasma containing a known factor activity
- Buffer to dilute patient plasma

A one-stage assay is termed as such because after preparation of this mixture, a single step of recalcifying the mixture leads to clot formation. The assessment of the quantity of factor activity is achieved by comparing the patient results in seconds to known activity of a specific factor. This is obtained by constructing a calibration curve using calibration plasma (see Appendix A). The calibration plasma is diluted with a physiological buffer into various levels of activity. Each dilution has a factor assay performed in which the endpoint detection represents the known standard factor activity in seconds. Results are

## The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines, which facilitates project management; defines a document structure using a template; and provides a process to identify needed documents. The QMS approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, 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 QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

H48 covers the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
QMS01	QMS01	GP41 M29 QMS01	QMS01	GP41	H57	X C24 EP05 EP06 EP09 EP10 EP15 EP17 EP26 EP28 GP41 H21 H47 H57	QMS01	QMS01	QMS01	EP10 H57	QMS01

### Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

H48 covers the medical 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 Reference Materials section on the following page.

	Preexamination				Examination			Postexamination	
	Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
GP41	X GP41 H21	X GP41 H21	X GP41 H21 H47	X GP41 H47	X GP41 H47 H57	X GP41	X	X	
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01

## Related CLSI Reference Materials\*

- C24**      **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 3rd ed., 2006.** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.
- EP05**      **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.
- EP06**      **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 1st ed., 2003.** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.
- EP09**      **Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed., 2013.** This document addresses the design of measurement procedure comparison experiments using patient samples and subsequent data analysis techniques used to determine the bias between two *in vitro* diagnostic measurement procedures.
- EP10**      **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. 3rd ed., 2014.** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.
- EP15**      **User Verification of Precision and Estimation of Bias. 3rd ed., 2014.** This document describes the estimation of imprecision and of bias for clinical laboratory quantitative measurement procedures using a protocol that can be completed within as few as five days.
- EP17**      **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.
- EP26**      **User Evaluation of Between-Reagent Lot Variation. 1st ed., 2013.** This document provides guidance for laboratories on the evaluation of a new reagent lot, including a protocol using patient samples to detect significant changes from the current lot.
- EP28**      **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. 3rd ed., 2010.** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- GP41**      **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture. 6th ed., 2007.** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.
- H21**      **Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. 5th ed., 2008.** This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storing plasma for coagulation testing; and general recommendations for performing the tests.
- H47**      **One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test. 2nd ed., 2008.** This document provides guidelines for performing the PT and APTT tests in the clinical laboratory, for reporting results, and for identifying sources of error.
- H57**      **Protocol for the Evaluation, Validation, and Implementation of Coagulometers. 1st ed., 2008.** This document provides guidance and procedures to the end user and manufacturer for the selection, evaluation, validation, and implementation of a laboratory coagulometer.

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\* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

## Related CLSI Reference Materials (Continued)

- M29**            **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US 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.
- QMS01**        **Quality Management System: A Model for Laboratory Services. 4th ed., 2011.** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.
- QSRLDT**      **Quality System Regulation for Laboratory-Developed Tests: A Practical Guide for the Laboratory. 1st ed., 2015.** This practical guide, compiled with the help of experts from the *in vitro* diagnostics industry, is intended for the laboratory that is creating laboratory-developed tests that may be subject to the US Food and Drug Administration (FDA) regulations, specifically the Quality System Regulation (QSReg), 21 CFR Part 820.

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