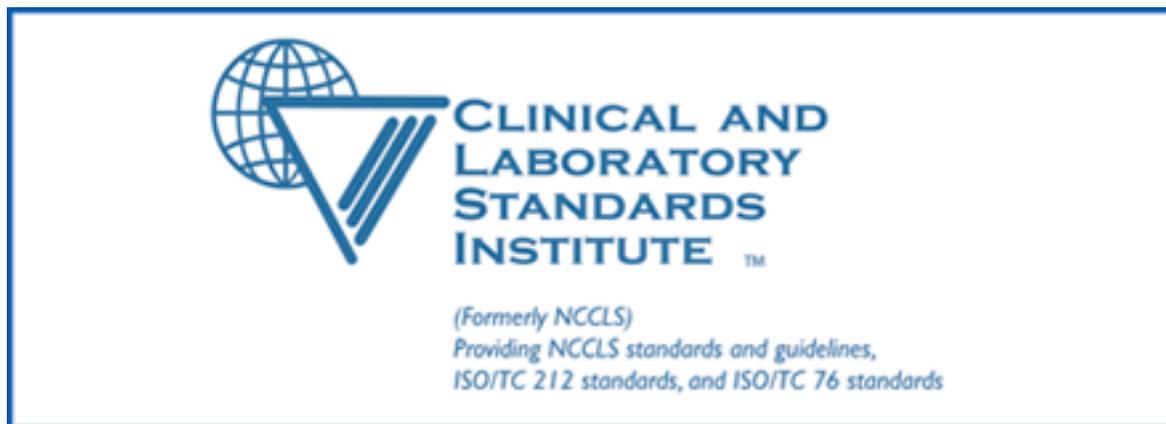

Procedure for the Determination of Fibrinogen in Plasma;
Approved Guideline—Second Edition



This document provides guidance on performing the fibrinogen assay in the clinical laboratory. Topics addressed include reporting of results and *in vivo* and *in vitro* conditions that may alter results.

A guideline for global application developed through the NCCLS consensus process.



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Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition

Abstract

Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition (NCCLS document H30-A2) is a performance guideline for laboratory and/or clinical healthcare professionals responsible for the routine performance of fibrinogen assays. This guideline describes a technique, based on the method described by Clauss,¹ that is practical, precise, and widely used in the clinical laboratory. Preanalytical and analytical factors and conditions that may alter results are discussed.

NCCLS. *Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition*. NCCLS document H30-A2 (ISBN 1-56238-439-2). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2001.

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Foreword

One of the major physiological roles of the coagulation system is to stop the loss of blood by generating a fibrin meshwork at the site of trauma or injury by converting the plasma glycoprotein fibrinogen to the fibrin polymer mesh. Numerous pathological conditions can induce decreased levels of fibrinogen rendering the coagulation system unable to generate sufficient fibrin to halt the blood loss. Increased levels of fibrinogen have been associated with cardiovascular disease and thrombosis. Decreased or increased levels of fibrinogen are clinically relevant and must be accurately determined. Laboratories should establish a normal reference interval for fibrinogen measurements. Generally, the normal reference interval is 150 to 350 mg/dL (1.5 to 3.5 g/L).

Fibrinogen, a β -globulin, is deficient in congenital afibrinogenemia and hypofibrinogenemia; in some cases of dysfibrinogenemia; and in a variety of acquired states, such as disseminated intravascular coagulation, systemic hyperfibrinolysis, severe hepatic dysfunction, and after treatment with L-asparaginase or sodium valproate. Spontaneous bleeding is usually not seen in patients with selected hypofibrinogenemia, i.e., fibrinogen levels of 50 to 100 mg/dL (0.5 to 1.0 g/L); however with certain types of hemostatic stress (surgery, trauma) bleeding may occur at levels up to 100 mg/dL (1.0g/L).

Fibrinogen is an acute phase reactant (i.e., a variety of physiologic stimuli or stresses such as pregnancy, inflammatory states, or estrogen use cause elevation of the plasma fibrinogen). Sustained progressive increases in fibrinogen within the upper 50th percentile of the normal reference interval or persistently elevated fibrinogen levels have increased risk of arterial and venous thrombosis (a prethrombotic state or a hypercoagulable state). Elevated fibrinogen levels have been implicated as a possible risk factor for the development of arterial and venous thrombotic complications.²⁻¹¹ Consequently, fibrinogen levels may be more widely used in the future for the assessment of the risk of developing thrombotic complications.

A number of assays of plasma fibrinogen have been described which are based on measurements of total clottable fibrinogen by protein assay,^{7,8} changes in turbidity or light scattering,⁹ salt precipitation,¹⁰ and fibrinogen antigen by various immunologic methods.^{2,11}

This document also describes a specific technique for the determination of fibrinogen concentration based on the Clauss thrombin clotting rate assay.¹ This assay is practical, precise, and widely used in the clinical laboratory. This document is primarily directed toward laboratory and/or clinical personnel responsible for obtaining and processing blood specimens, performing the fibrinogen assay and quality control procedures, and reporting fibrinogen assay results. It is also intended as a guide for manufacturers of the reagents and instruments. Preanalytical and analytical factors and conditions that may alter results are discussed.

Other international standards procedures have been established for determination of fibrinogen in plasma. This guideline was separately from standards for fibrinogen determination prepared by the Deutsches Institut für Normung (DIN).^{12,13} On comparison, the documents appear to be essentially the same. Some differences do occur based on available and standard reagents. Use of either should allow determination of fibrinogen concentration. NCCLS will work toward harmonization in the next revision of H30.

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; aids in defining document structure; and provides a process to identify needed documents through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS document [GP26](#)—*A Quality System Model for Health Care*.

Foreword (Continued)

The quality system approach applies a core set of “quality system essentials,” basic to any organizational process, to all operations in the healthcare service’s path of workflow. The quality system essentials are: organization; personnel; equipment; purchasing and inventory; process control; documents and records; occurrence management; internal assessment; process improvement; service and satisfaction; facilities and safety; and information management. The path of workflow for the clinical laboratory consists of three process areas: preanalytical; analytical; postanalytical.

NCCLS document H30-A2—*Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition* describes the preanalytical and analytical aspects of the path of workflow for the clinical laboratory by specifically providing guidance on the collection, transportation, handling, and storage of the specimen or sample and on performing fibrinogen assays in the laboratory.

Key Words

Afibrinogenemia, Clauss method, disseminated intravascular coagulation (DIC), dysfibrinogenemia, fibrin, fibrinogen, hypofibrinogenemia

Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition

1 Introduction

This document specifies a technique to assay fibrinogen in plasma, based on the method of Clauss.¹

2 Scope

H30-A2 contains guidelines for the collection, transportation, handling, and storage of blood specimens or plasma samples and general guidelines for performing the fibrinogen assay by the Clauss method.¹ It is primarily directed toward laboratory and/or clinical personnel responsible for obtaining and processing blood specimens, performing the fibrinogen assay and quality control procedures, and reporting fibrinogen assay results. It is also intended as a guide for manufacturers of the reagents and instruments. The guideline does not cover prothrombin-time (PT)-derived fibrinogen determination which can be performed using various automated coagulation instruments.¹⁴

3 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document [M29—Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue](#).

4 Definitions^a

Control (plasma), *n* - A batch of citrated plasma used to monitor the stability of the laboratory test system, which includes reagents, instruments, reconstituting and diluting fluids, and pipettes; **NOTES:** a) “Normal control plasma” gives test results within the range of the reference interval; b) “Abnormal control plasmas” for factor assays should contain factor concentrations below the reference interval values due to abnormally low factor concentrations; c) If factors are clinically elevated, the “abnormal control plasma” should contain factor concentrations above the reference interval; d) Normal and abnormal control plasmas may be prepared in the laboratory or obtained commercially.

Reference curve, *n* - A line, typically a straight line, that defines the quantitative relationship between an independent variable and a dependent variable; **NOTE:** From this line the observed output of an analytic procedure (e.g., APTT test) can be converted to the units of measurement of the analyte of interest (e.g., coagulation factor activity).

^a Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

Reference plasma, *n* - Citrated normal pooled plasma of known coagulant factor activity prepared in-house or available from a manufacturer; **NOTE:** This plasma is used to construct the reference curve.

Sample (patient), *n* - A sample taken from the patient specimen and used to obtain information by means of a specific laboratory test.

Specimen (patient), *n* - The discrete portion of a body fluid (e.g., blood) or tissue taken for examination, study, or analysis of one or more quantities or characteristics to determine the character of the whole.

Test plasma, *n* - Plasma (from a patient or unknown source) that is analyzed by a specific laboratory test.

5 Principle

In normal blood coagulation, fibrinogen is converted to fibrin in a two-step process. The first step is the thrombin-mediated limited proteolysis of two small peptides from fibrinogen-creating fibrin monomers. In the second step, these monomers (when present in sufficient concentration) spontaneously polymerize to form fibrin strands or polymers. The formation of fibrin polymers is the end point of the fibrinogen assay which is used to calculate fibrinogen concentration. In normal plasma, fibrin polymers are rapidly cross-linked by thrombin-activated factor XIII (fibrin stabilizing factor) to form insoluble fibrin. However, this reaction is not measured in the fibrinogen assay.

The most commonly used method for the determination of fibrinogen concentration is the Claus thrombin clotting rate assay described in this document. A standard amount of thrombin is added to diluted plasma, and the time required for clot formation is recorded. The clotting time of dilute, citrated plasma is inversely proportional to the fibrinogen concentration of the plasma when a relatively high concentration of thrombin is used. The clotting time obtained is then compared with the clotting times of a series of diluted reference plasma of known fibrinogen concentration to yield the fibrinogen concentration of the test plasma.

The standard method recommended by DIN is essentially the same as the method described herein. Some differences do occur based on differences between available and standard reagents.^{12,13}

6 Equipment

6.1 Containers

Using a semiautomated or automated end point detection system, the test is performed using nonactivating surface containers.

6.2 Delivery Systems

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

6.3 Heating Block

A heating block or water bath should be available to preheat and maintain reagents at 37 ± 1 °C.

7 Specimen Handling

7.1 Patient Information

A test requisition (computer order or hard copy) should accompany all specimens and should include patient demographic information, a unique patient identification number, and indication for testing. Pertinent patient information, such as treatment with heparin or warfarin, should be indicated as clinical information when appropriate and possible.

7.2 Specimen Collection, Transportation, Processing, and Storage

Blood should be collected, transported, processed, and stored according to the most current edition of NCCLS document [H21](#)— *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays*.

7.3 Post-test Specimen Management

Please refer to the most current edition of NCCLS document [M29](#)—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue* for recommendations on specimen disposal and medical waste management.

8 Reagents

8.1 Commercial Reagents

There are several commercial reagent systems that are widely used for this assay. If one of these systems is used, the manufacturer's recommendations and instructions for reagents, procedures, and performance of the test should be strictly followed.

8.2 Non-Commercial Reagents

If noncommercial reagents are to be used in the determination of fibrinogen, the reagent preparations listed below are recommended.

8.2.1 Barbitol Buffer (Owren's buffer; 0.02 mol/L, pH 7.4 ± 0.05)

To a 2-L volumetric flask, approximately 1 L of Type I distilled water ([Section 12.2](#)), 430 mL of 0.1 mol/L HCl, 11.7 g sodium diethylbarbiturate, and 14.7 g sodium chloride are added, and the mixture is stirred until complete dissolution is achieved. A sufficient volume of water is added to bring the volume to the 2-L mark. The pH of the buffer at 25 °C is adjusted to pH 7.4 with 1N HCl. The buffer is stable for at least three months when stored at 4 °C. Equivalent buffers may be used.

8.2.2 Thrombin

Thrombin (bovine or human), of known National Institutes of Health (NIH) units and prepared in barbitol buffer, should be kept in a lyophilized form or frozen in aliquots in nonactivating surface containers. A frozen thrombin stock solution of 1,000 NIH units/mL is stable for at least one year at -70 °C. The stability of frozen thrombin at temperatures above -70 °C has not been well documented; however, 98% of clinical laboratories use commercially available lyophilized thrombin preparations with well-documented stability and clearly marked expiration dates. The expiration date criteria specified by the manufacturer should be strictly followed. (If thrombin is stored in a frozen state, then it is extremely important to aliquot the working thrombin solution in volumes appropriate for individual assay runs,

because repeated freeze-thaw cycles will denature the enzyme.) Before use, aliquots of thrombin should be reconstituted or thawed, the proper dilution should be made in barbital buffer, and it should be kept at 2 to 8 °C until needed for the test. Thrombin in working dilutions is typically stable for no more than one hour at 2 to 8 °C.

8.2.3 Normal Plasma Control

Normal plasma controls can be derived from a pool of commercial citrated normal plasmas, or prepared in the laboratory and stored in aliquots. If lyophilized, this material should be kept at 2 to 8 °C until the expiration date and, if frozen, stored in aliquots at ≤ -20 °C for up to six months. An aliquot of pooled normal plasma should be thawed or reconstituted and tested at the usual dilution used for normal plasma controls with each aliquot of reconstituted working thrombin solution. The measured fibrinogen concentration in thawed or reconstituted plasma is stable for six hours at a temperature of 2 to 8° C.

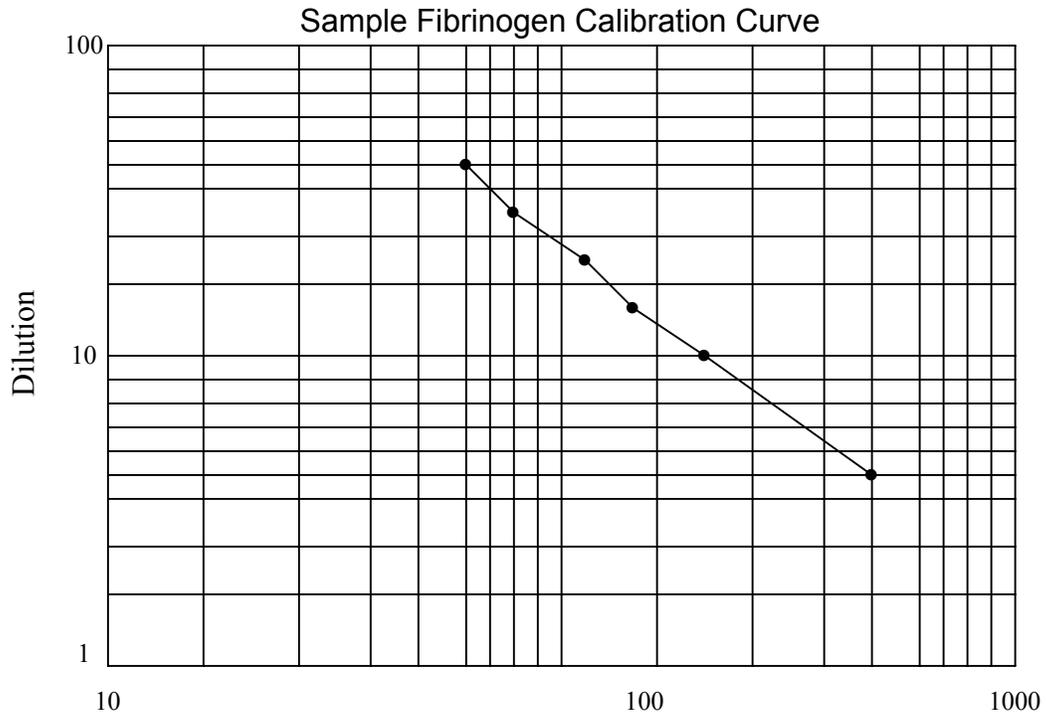
8.2.4 Abnormal Control

At least one abnormal control with a decreased fibrinogen level [80 to 100 mg/dL (0.8 to 1.0 g/L)] should also be assayed with every batch of specimens tested. In laboratories where many fibrinogen determinations are performed, normal and abnormal controls should be tested at a minimum of every 20 patient samples. Laboratories should follow the kit manufacturers' recommendations. An aliquot should be thawed or reconstituted and tested at the usual dilution used for normal plasma controls with each aliquot of reconstituted working thrombin solution. Commercial abnormal controls are available. Abnormal controls can be prepared by diluting pooled normal plasma with barbital buffer. The prepared abnormal control pool should be assayed, aliquoted, and stored at ≤ -20 °C.

9 Reference Curve

The reference curve should be prepared from reference plasma calibrated against a standard plasma with a known fibrinogen concentration. Reference plasmas may differ from those reported in the DIN procedures.^{12,13} Reference plasmas are available from the World Health Organization and coagulation reagent manufacturers.¹⁵ The manufacturer's recommendations and instructions should be followed. New reference curves should be prepared with each change of reagent lots, any change of instrument, or with any deviation from quality control or proficiency testing limits. Calibration should be performed on each new lot of pooled reference plasma.

At least five points are recommended in the construction of the reference curve within the acceptable measuring range. The reference curve is prepared by plotting the logs of the clotting times of reference plasma dilutions against the logs of the fibrinogen concentrations. An example of a reference curve is shown in [Figure 1](#). At least five points should be used in the construction of the reference curve. The fibrinogen concentration in diluted test plasma is read from the reference curve.



Dilution	Concentration (mg/dL)	Mean Clotting Time (sec)
1:5	476	5.4
1:10	238	9.8
1:15	159	14.9
1:20	119	21.7
1:30	79	31.3
1:40	60	47.1

Figure 1. Curve for Quantitative Fibrinogen Procedure

10 Procedure

- (1) Collect blood into citrate anticoagulant according to the method described in the current edition of NCCLS document [H21—Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays](#).
- (2) Treat control and patient plasma in an identical manner. Dilute the plasma 1:10 with barbital buffer (see [Section 8.2.1](#)). Place the diluted plasma in the reaction container and warm to 37 ± 1 °C for five minutes.
- (3) Prepare thrombin working solution by diluting stock thrombin [of known NIH units] with barbital buffer (see [Section 8.2.2](#)) to the desired concentration. The thrombin working solution must not be frozen before use.
- (4) Add 0.1 mL of thrombin working solution to 0.2 mL of diluted test plasma, and simultaneously start a timer.
- (5) Read the values of the fibrinogen concentration in mg/dL from the reference curve, and multiply by the appropriate plasma dilution to obtain the final mg/dL value.
- (6) If the clotting time falls within the linear portion of the reference curve, read the result directly from the curve. If the thrombin clotting time is outside the linear portion of the curve, repeat the test using different test plasma/buffer dilutions until a clotting time is obtained that falls within the linear portion of the curve. When thrombin clotting times below the linear portion of the curve are obtained, a greater dilution (e.g., 1:20 to 1:40) is used. When thrombin clotting times are above the linear portion of the curve, a lower dilution (i.e., 1:5 or 1:3) is used. The lowest dilution that may be used with any degree of acceptable accuracy is 1:3; undiluted plasma cannot be used, because inaccurate results may be obtained due to the presence of interfering substances.

When the plasma fibrinogen concentration is below the detectable range with a 1:3 plasma dilution, the result should be reported as less than the value determined by the greatest dilution on the linear portion of the reference curve (corrected for the dilution of the patient sample).
- (7) Although NCCLS documents generally use units that are fully acceptable within the *Système International d'Unités* (SI), these do not always coincide with the units recommended by the International Union of Pure and Applied Chemistry (IUPAC) and by the International Federation of Clinical Chemistry (IFCC) for reporting results of clinical laboratory measurements.¹⁶ SI units are used worldwide, but there is not yet a consensus as to their use in the United States; NCCLS documents include the IUPAC/IFCC recommended units of volume (L) and substance (molecular) concentration (mol/L) in parentheses, where appropriate. Because of the uncertainty in the value of the relative molecular mass of fibrinogen, IFCC-IUPAC recommends that results be reported in grams per liter.

11 Test Result Management

Users should follow institutional policy for entering patient test results into the existing laboratory information system (LIS) to minimize/eliminate clerical errors and to ensure prompt and accurate result reporting.

Low critical values should be established by the laboratory and a procedure put in place for communicating these critical values to the appropriate clinical staff caring for the patient. Notification of the critical value (date/time/individual notifying/notified individual) should be documented.

12 Considerations in Performing the Fibrinogen Assay

12.1 Manufacturers' Instructions

Manufacturers' instructions for reagents and equipment should be strictly followed.

12.2 Water

Type I reagent grade water should be used (see the current edition of NCCLS document [C3](#)—*Preparation and Testing of Reagent Water in the Clinical Laboratory*).

12.3 Cleaning

All collection and storage tubes, pipets, and delivery systems should be clean.

12.4 Temperature

The test should be performed at 37 ± 1 °C.

12.5 End Point Determination

The end point can be measured by a variety of optical or electromechanical methods.

12.6 Quality Control

The laboratory should follow generally accepted quality control practices and quality control requirements of the appropriate regulatory agencies. Specifically, laboratory personnel with appropriate experience in performing fibrinogen assays should inspect the quality control results to evaluate for trends or shifts, as well as out-of-limit results. In addition, the plots of the reference plasmas and the individual patient/test plasmas should be reviewed for correctness. There should be periodic review of quality control data to look for long-term changes in the analytic system.

12.6.1 Controls Outside Stated Limits

If the test values for the control plasmas are not within stated limits, all reagents, control plasmas, and equipment should be checked. The actions undertaken to identify and correct the problem should be documented before any patient plasma is analyzed in the system.

12.7 Reference Interval

Each laboratory should develop its own reference interval for the fibrinogen assay.¹⁶ The details of the procedures for determination of reference intervals for coagulation proteins can be found in the current edition of NCCLS document [H21](#)—*Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays*.

13 Sources of Preamalytical Error

13.1 Inappropriate Specimen Collection

Problems of inappropriate specimen collection include the following:

- incorrect collection tube (i.e., lack of anticoagulant or incorrect anticoagulant, e.g., heparin, EDTA);
- overfill or underfill of collection tubes;
- failure to adjust the citrate volume for persons with very high (>0.55) hematocrit (packed cell volume) (see the current edition of NCCLS document [H21](#)—*Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays*);
- clotted, hemolyzed, icteric, or lipemic specimens;
- inadequate or too vigorous agitation of a specimen; or
- contaminated collection or storage tubes.

14 Sources of Analytical Error

14.1 Inappropriate Thrombin Preparation

Problems with thrombin solution may include:

- contaminated buffer or reagent;
- reconstitution with an incorrect volume of buffer;
- use of thrombin working solution after freezing;
- defects in the commercial thrombin reagent;
- storage in glass; or
- prolonged (> 1 hour) storage or storage at ≥ 8 °C of thrombin at working solution dilution.

14.2 Incorrect Conditions

Incorrect conditions that may affect test results include using the wrong incubation time, temperature, buffer pH, volumes, or instrumentation procedures.

14.3 Paraproteins

High levels of some paraproteins may interfere with the polymerization of fibrin monomers, leading to underestimation of fibrinogen.

14.4 Bovine Thrombin Antibodies

The clinical use of topical bovine thrombin may lead to the development of antibodies to thrombin. These antibodies may lead to artifactual reduction in the rate of thrombin formation and underestimation of fibrinogen.

14.5 Fibrin/Fibrinogen Degradation Products (FDP)

Proteolytic products of fibrinogen and fibrin in high concentration may interfere with fibrin polymerization. At fibrinogen concentrations below 150 mg/dL (1.5 g/L), FDP greater than 75 µg/mL (75 mg/L) decrease the rate of fibrin polymerization and underestimate fibrinogen concentration.¹⁷

14.6 Heparin

Heparin, a potent activator of antithrombin, which irreversibly inhibits clotting-active serine proteases including thrombin, may interfere with the thrombin clotting time, thus causing erroneously low estimates of fibrinogen concentration. It may require higher concentrations of heparin (greater than 5.0 U/mL) to accomplish this inhibition.¹⁷ Although these concentrations are infrequently seen in the clinical use of heparin, these concentrations may be found in blood that is collected incorrectly in heparinized tubes or through heparinized lines (high local concentration), and in patients undergoing cardiopulmonary bypass surgery, or during hemodialysis.

14.7 Dysfibrinogenemia

In certain patients with acquired or inherited biochemical abnormalities of fibrinogen which may inhibit the action of thrombin on fibrinogen and/or fibrin polymerization (i.e., dysfibrinogenemia), fibrinogen levels may be underestimated.

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NCCLS consensus procedures include an appeals process that is described in detail in Section 9.0 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

H30-A: *Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline*

Section 8 (now Section 10)

1. “The difference between duplicate results should agree within 7% of their mean value.” This is a change from the H30-T (1991) which states, “Duplicate results should agree with a CV <7% for the mean of duplicates.” A CV of 7% equates to a difference of 10% between duplicate results relative to their mean. I am not sure if there was an error in trying to clarify this section or if there was a decision to apply a more stringent criterion. In my experience, the fibrinogen assay gives higher CVs than either PT or PTT duplicates which NCCLS has assigned a 10% between duplicates (see H47, Section 7.9). I believe that a 10% agreement between duplicates is acceptable and indeed was probably the intent of the document. Requiring 7% agreement causes a number of needless reruns of clinically valid data when a 10% agreement would provide adequate assurance of duplication of results.
- **The subcommittee has deleted references to acceptable differences between duplicate measurements. Differences between duplicate results should be determined by the user. Recommendations regarding acceptable imprecision of the procedure have been included.**

Summary of Delegate Comments and Subcommittee Responses

H30-A2: *Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition*

General

1. Please note that the correct affiliation for Dr. Brandt is now Eli Lilly & Co., Indianapolis, Indiana.
 - **This editorial correction has been made to the “Committee Membership” page.**
2. We are disappointed that the content of this document is limited to Clauss-based fibrinogen assays, and does not address PT-derived fibrinogen assays.
 - **PT-derived (i.e., calculated) methods for fibrinogen were not available at the time H30-A2 was developed. The committee will address PT-derived assays in the next version of the document.**
3. The Deutsches Institut für Normung (DIN) has put forth recommendations regarding the fibrinogen standard and fibrinogen assay. DIN document 58939 provides a description of the fibrinogen reference plasma calibrated against WHO standard. DIN document 58906 provides the standard procedures for fibrinogen assay in plasma. In DIN 58906, they describe more specifically the method for fibrinogen determination by a thrombin-derived assay. These documents should be considered in the description of the NCCLS document.
 - **The subcommittee appreciates the commenter’s suggestion. H30-A2 has been compared with DIN 58939 and DIN 58906. References to the DIN publications are included where appropriate.**

Foreword

4. The Foreword needs editing to make it more user friendly to readers who are not coagulation experts. Things as simple as a sentence following the first sentence in the first paragraph that would explain that sometimes the fibrinogen level is too low to do its job and that sometimes it is too high. That’s why it is important to be able to measure it. Then you could start a new paragraph with the existing material regarding deficiencies, followed by a paragraph on the thrombus problem.
 - **The subcommittee has rewritten the Foreword to include a general discussion of the physiologic role(s) of fibrinogen.**
5. Make the insert of the transportation information more seamless. One could argue this belongs in the Quality Systems part to explain that NCCLS has another document that addresses another element in the path of workflow.
 - **The subcommittee has rewritten the Foreword. Information on collection, transportation, handling, and storage has been incorporated into the “Quality System Approach” discussion.**
6. The authors discuss the characteristics of fibrinogen as an acute phase reactant and as a potential marker of risk factor for arterial and venous thromboembolic problems. Is there a component of persistence that should be mentioned with the risk of thromboembolic disease? As the paragraph reads now, it could be construed that a transient increase in fibrinogen as an acute phase reactant may be a risk factor for vascular disease and thromboembolic disease.

- **The text has been revised to address persistently increased levels of fibrinogen and the relationship to thrombotic risk.**
7. In the first paragraph, provide a normal value (in mg/dL) for fibrinogen.
- **An estimated range for normal values has been included.**
8. In the last sentence of the first paragraph, I suggest rewording the example to read, “i.e., fibrinogen levels under 50 mg/dL (0.5 g/L); however, *under* certain types of hemostatic stress (surgery, trauma) bleeding may occur at levels *under* 100 mg/dL (1 g/L).
- **The text has been revised to address the comment.**

Section 4

9. The documents indicate that abnormal control plasmas should contain factor levels below the reference interval. In view of the increasing interest in increased levels of clotting factors, including fibrinogen, would it not be appropriate to consider adding abnormal control plasmas with increased levels of factors?
- **A note has been added to the definition, taking into account the increased nature of some factors.**

Section 8.2.4

10. We think recommending quality control per a minimum of 20 samples in laboratories where many fibrinogen determinations are performed is excessive. If quality control is performed each shift and reliable, automated instrumentation is utilized, a larger number may be acceptable. We suggest adding the statement to directing the user to follow the manufacturer’s recommendations. For example, the manufacturer of our system recommends that controls be analyzed after four hours or 100 specimens.
- **The subcommittee recommends performing quality control per a minimum of 20 samples. Text has been added suggesting laboratories follow the kit manufacturer’s recommendations.**

Section 9

11. In the draft, it is recommended to calibrate an instrument that “At least five points should be used in the construction of the reference curve.” Currently in Japan, about 70% of laboratories are using automated instruments that automatically run measurements using a calibration curve constructed by three (3) points diluted from one calibrator (1:5, 1:10, and 1:20). However, Japanese manufacturers have developed new technology that allows the user to select the dilution ratio for the calibration. Up to six (6) points can be selected and programmed for construction of the reference curve.

Despite provision of an example of the curve produced by the dilutions of 1:5, 1:10, 1:15, 1:20, 1:30, and 1:40, it is not precisely mentioned that users should select the dilution ratio from 1:5 to 1:40. It is understandable that in selecting the dilution ratio one must consider the concentration of a calibrator and the measuring range. The descriptions of these conditions should be clarified.

Japanese manufactures are willing to recommend the NCCLS procedure described in H30-A2. We request that the following description is added for clarification: “At least five (5) points are recommended in the construction of the reference curve within the acceptable measuring range.”

- **The subcommittee believes that a five-point curve is necessary for calibrating the fibrinogen assay. Studies must be conducted to validate the use of a three-point curve. Also, manufacturers recommendations should be followed. The suggested description has been added for clarification.**

Related NCCLS Publications*

- C3-A3** **Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline—Third Edition (1997).** This document provides guidelines on water purified for clinical laboratory use; methods for monitoring water quality and testing for specific contaminants; and water system design considerations.
- C28-A2** **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (2000).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- H1-A4** **Evacuated Tubes and Additives for Blood Specimen Collection—Fourth Edition; Approved Standard (1996).** American National Standard. This standard contains requirements for blood collection tubes and additives including heparin, EDTA, and sodium citrate.
- H3-A4** **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard— Fourth Edition (1998).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.
- H21-A3** **Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays; Approved Guideline—Third Edition (1998).** This guideline contains procedures for collecting, transporting, and storing blood, processing blood specimens, storing plasma for coagulation testing, and provides general recommendations for performing the tests.
- H47-A** **One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline (1996).** This document provides guidelines for performing the PT and APTT tests in the clinical laboratory, for reporting results, and for identifying sources of error.
- H48-A** **Determination of Factor Coagulant Activities; Approved Guideline (1997).** A consolidation of Factor VIII and Factor IX assays guidelines, this document addresses the performance, quality control, and reporting of assays for coagulation factor activity based upon conventional APTT and PT coagulation tests.
- M29-A** **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document offers guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in the laboratory; gives specific precautions for preventing transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

Related NCCLS Publications (Continued)

NRSCL8-A Terminology and Definitions For Use in NCCLS Documents; Approved Standard (1998). This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

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