Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition

This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storage of plasma for coagulation testing; and general recommendations for performing the tests.

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

NCCLS document H21-A4—Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition is an update of the previous edition published in 1998. The guideline provides procedures for the collection, transport, and processing of blood specimens for coagulation testing. Tests of the coagulation system are very sensitive to storage (time and temperature), concentration of anticoagulant, and surface of containers; attention to these parameters is important. H21-A4 is primarily directed toward laboratory and/or clinical personnel responsible for obtaining patient specimens and preparing plasma for analysis by coagulation testing.


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Foreword

NCCLS document H21-A4 is part of a series of guidelines involving methodology in blood coagulation testing. Because of the many variables that can affect coagulation test results, the Subcommittee on Coagulation concluded that it would be advantageous to provide a guideline which describes procedures for collection, storage, and preparation of blood or plasma before and during coagulation testing. This publication should increase the uniformity of coagulation testing and, thereby, reduce many variables that can affect the test results. Other publications in the series deal with specific coagulation assays such as the prothrombin time test, the activated partial thromboplastin time test, the fibrinogen assay, coagulation factor assays, and bleeding time test, as well as other assays.

The working group wishes to thank all commenters for their recommendations on the third-edition approved guideline (H21-A3). The Area Committee on Hematology urges users to submit comments related to experience in using H21-A4 to assure future editions reflect the “state of the art.” Each comment will be carefully reviewed, and changes will be made where appropriate. NCCLS is dedicated to quality clinical laboratory services, and this guideline covers one of the many areas in which recommendations are being developed to help achieve this end.

This document replaces the third edition approved guideline, H21-A3, which was published in 1998. Several changes have been made in this edition; chief among them is the revised platelet count limit for the platelet poor plasma used in PT, APTT, and TT tests (Section 5.1). This guideline also contains revised recommendations regarding specimen storage (Section 5.2). The recommendations regarding the collection of coagulation specimens (Section 4.1) have been revised for consistency with NCCLS document H3—Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard.

Key Words

Activated partial thromboplastin time, citrate, coagulation, coagulation factors, control (plasma), fibrinogen, prothrombin time, sample storage, specimen collection, specimen transport, thrombin time
Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition

1 Scope

This guideline covers the procedures for the collection, transport, and processing of specimens for coagulation tests. Many variables, including anticoagulant amount and concentration, specimen and sample storage, and surface of containers, may affect test results. The document is directed toward laboratory and/or clinical personnel responsible for obtaining patient specimens and preparing plasma for analysis by coagulation testing. It is also aimed at manufacturers of products involved in specimen collection, storage, and preparation for coagulation testing. This document does not address whole blood clotting tests or point-of-care testing. In addition, H21-A4 provides general guidelines for performance of coagulation testing. Performance guidelines for specific coagulation assays are addressed in other NCCLS documents, such as those for PT and APTT assays (i.e., H47—One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test) and fibrinogen assay (i.e., H30—Procedure for the Determination of Fibrinogen in Plasma).

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 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 for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—Protection of Laboratory Workers from Occupationally Acquired Infections.

2 Introduction

A procedural guideline for the collection, transport, and processing of specimens for coagulation tests is necessary, as many variables may affect test results (e.g., concentration and amount of anticoagulant, specimen and sample storage time and temperature, the surface of containers in which the specimen is obtained and stored). Because important diagnostic and therapeutic decisions are based on the results of coagulation tests, a procedural guideline for the collection, transport, and processing of blood specimens for the general performance of coagulation assays is warranted.

3 Definitions

Activated partial thromboplastin time (APTT) – The time, in seconds, required for a fibrin clot to form in a plasma sample after appropriate amounts of calcium chloride, a partial thromboplastin reagent, and a wettable surface have been mixed with the sample; NOTE: The APTT measures the intrinsic and common coagulation pathways.
Activated partial thromboplastin time test (APTT) – A measure of the time required for the formation of fibrin under specified conditions (e.g., partial thromboplastin, Ca); NOTE: It is used for the evaluation of the intrinsic and common coagulation pathway and for monitoring therapy with unfractionated heparin (but not low molecular weight heparins) and certain other anticoagulants.

Anticoagulant – An agent that prevents coagulation of blood.

Blood collection device – An evacuated tube, syringe, or other device with nonactivating surface.

Blood collection system – A system consisting of several components, such as catheter, connecting device, syringe, needle, and collection device, used for blood collection.

Coagulation factors – The various components of the blood coagulation system; NOTE: The following factors (including synonyms which are, or have been in use) have been identified:

Factor I (fibrinogen)
Factor II (prothrombin)
Factor III (commonly termed thromboplastin, tissue factor)
Factor IV (commonly termed calcium)
Factor V (labile factor)
Factor VII (stable factor)
Factor VIII (antihemophilic factor [AHF], antihemophilic globulin [AHG], antihemophilic 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]).

Control plasma – A preparation of fresh, frozen or lyophilized plasma collected from human or animal blood, or artificially derived material, intended for use in the quality control process; NOTES: a) Control plasmas are used to monitor all aspects of the laboratory test system, including the reagents, instruments, reconstituting and diluting fluids, and pipets; b) Normal controls should give test results within the reference interval; c) Abnormal control plasmas should give values within the clinically relevant abnormal range.

Dead space volume – The volume of blood that would fill the length of a catheter lumen; NOTE: This term is used in the collection of blood from indwelling catheters.

Fibrinogen assay – The assay of fibrinogen concentration; commonly measured by the rate at which it is converted to fibrin by the action of thrombin; NOTE: It is described in NCCLS document H30—Procedure for the Determination of Fibrinogen in Plasma. Other methodologies include precipitation/gravimetric, immunological and nephelometric procedures.

International normalized ratio (INR) – Expression of the patient’s prothrombin time (PT) test result expressed as a ratio to a normal population control which has been standardized (or normalized) for the potency to the thromboplastin used in the assay [ISO/CD 17593]¹; NOTE: It is standardized using a World Health Organization (WHO) international reference thromboplastin preparation, and determined using the equation: INR = R\(^{0.04}\), where R is the PT ratio obtained with the working thromboplastin.

International sensitivity index (ISI) – A mathematical indicator of the responsiveness of a PT testing system to deficiencies of the Vitamin K coagulation factors; NOTES: a) It is the comparative slope used
to calculate the INR (see definition above); b) A low ISI indicates a highly responsive PT system and a high ISI indicates a poorly responsive system; c) It is determined by standard protocols according to WHO guidelines and provided by the manufacturer to the user for a particular reagent/instrument combination.

Nonactivating surface – A surface that does not activate coagulation factors (as indicated by lengthening or shortening of the PT or APTT).

Patient sample – A sample taken from the patient specimen and used to obtain information by means of a specific laboratory test.

Patient specimen – The discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more qualities or characteristics, to determine the character of the whole; NOTE: For coagulation testing, the specimen would be anticoagulated blood or plasma.

Prothrombin time (PT//Time of tissue factor-induced coagulation) – Time required to clot a blood specimen once exposed to a thromboplastin reagent material (e.g., tissue factor and calcium chloride); [ISO/CD 17593].

Prothrombin time test (PT test) – A test used for evaluation of the extrinsic and common coagulation pathway and for monitoring oral anticoagulant therapy.

Reference interval – The range of test values expected for a designated population of individuals; NOTES: a) Typically, a population of healthy, age-matched individuals; b) For example, 95% of individuals that are presumed to be healthy or normal.

Thrombin time (TT) – The time in seconds required for a fibrin clot to form in a sample after a known amount of suitable thrombin has been added. (For additional information, please see the most current edition of the NCCLS document H30—Procedure for the Determination of Fibrinogen in Plasma.)

Thrombin time test (TT test) – A test used to evaluate the final steps of the coagulation pathway.

Vascular access device (VAD) – A device inserted into a vein or artery to allow access to the circulatory system for the administration of intravenous fluids and/or medications.

4 Specimen Collection

4.1 Methods for Obtaining Blood Specimens

It is recommended that blood specimens for coagulation testing be collected by venipuncture using a blood collection system that collects the specimen directly into a tube containing the anticoagulant. For proper venipuncture technique, see the most current edition of NCCLS document H3—Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture. All specimens should be collected in a nonactivating surface container.

Syringe draws using a hypodermic needle/syringe may have limitations because of the increased risk of hemolysis and apparent safety issues. With larger syringes, there is an increased chance that clotting may occur. If a syringe is used, a small-volume syringe (≤20 mL) is recommended. If a needle is used on the syringe to obtain the specimen, appropriate safety procedures should be employed in its removal. (See the most current edition of NCCLS document M29—Protection of Laboratory Workers from Occupationally Acquired Infections).
Under certain circumstances, blood specimens for coagulation testing may be drawn from a vascular access device (VAD) using a blood collection system or a syringe. When obtaining a blood specimen from a VAD, the components of the blood collection system (VAD, connecting device, syringe, needle, and collection device) should be checked to ensure compatibility to avoid air leaks which may cause hemolysis and incorrect draw volumes. Collection of the blood through lines that have been previously flushed with heparin should be avoided, if possible. If the blood must be drawn through a VAD, possible heparin contamination and specimen dilution should be considered. In this case the line should be flushed with 5 mL of saline and the first 5 mL of blood or six dead space volumes of the VAD discarded.2

Studies have shown that the PT (INR) and APTT results are not affected if tested on the first tube drawn.3-6 Proof of necessity of drawing a discard tube for other coagulation testing is circumstantial at best, but there are no current published data to suggest that this practice is unnecessary. For the appropriate order of draw when collecting multiple specimens, see the most current edition of NCCLS document H3—Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture. When using a winged blood collection set for venipuncture and a coagulation tube is the first tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection tubing dead space and to assure maintenance of the proper anticoagulant/blood ratio; it need not be completely filled. The discard tube should be a non-additive or a coagulation tube. If a double-syringe technique is used, blood from the second syringe should be used for the coagulation specimen. In the case of any unexplained abnormal coagulation test result, a new specimen should be obtained and the test repeated. If heparin contamination is suspected, the test should be repeated after the specimen is treated with a method that removes or neutralizes heparin.

When using the hypodermic needle/syringe, it is important that the blood is added to the appropriate volume of anticoagulant within one minute of completion of draw. Regardless of the device used for specimen collection, all tubes should be inverted at least four times to mix. Excessive mixing can cause hemolysis and/or platelet activation, leading to erroneous results.

### 4.2 Anticoagulant and Blood/Anticoagulant Ratio

#### 4.2.1 Anticoagulant

The anticoagulant used for coagulation assays should be 105 to 109 mmol/L, 3.13% to 3.2% (commonly described as 3.2%)7 of the dihydrate form of trisodium citrate (Na$_3$C$_6$H$_5$O$_7$ • 2H$_2$O), buffered or nonbuffered. Other anticoagulants (e.g., oxalate, heparin, or EDTA) are unacceptable.

#### 4.2.2 Blood/Anticoagulant Ratio

The proportion of blood to the sodium citrate dihydrate anticoagulant volume is 9:1. Inadequate filling of the collection device will decrease this ratio, and may lead to inaccurate results. 8-10 The manufacturer’s recommendations should be followed.

#### 4.2.3 Citrate Concentration Adjustments

The final citrate concentration in the blood should be adjusted in patients who have hematocrit values above 0.55 L/L (55%). For hematocrits below 0.20 L/L (20%), there are no current data available to support a recommendation for adjusting the citrate concentration. The chart in the appendix can be used to determine the amounts of anticoagulant and blood for hematocrit values above 0.55 L/L.
4.3 Needle Gauge

Manufacturers of needles and blood collection sets have developed automated manufacturing procedures designed to eliminate inner surface roughness, which may contribute to possible hemolysis and thrombogenicity. The more important issue facing the phlebotomist is the assessment of the subject prior to performing phlebotomy, which should include determination of the appropriate needle gauge to be used based on the amount of blood to be drawn, age of the subject, and size of his/her veins. If these points are considered, hemolysis and thrombogenicity due to preanalytical variables will be minimized. Additionally, winged blood collection sets, because of their longer path length between vein and anticoagulant, when used in combination with smaller-gauge needles should be used with caution to avoid platelet and coagulation activation.

5 Specimen Transport, Processing, and Sample Storage

5.1 Preparation of Suitable Plasma Specimens

Specimens that are clotted, collected in the wrong anticoagulant, or are in collection devices that have less than the recommended fill, are not suitable for testing and should be rejected. The whole blood specimen should be checked for clot formation by gentle inversion and observation. To obtain a plasma sample, the capped specimen tube should be centrifuged at a speed and time required to consistently produce platelet-poor plasma (platelet count < 10 x 10^9/L) (10,000/µL). This may be accomplished by centrifuging at 1,500 g for no less than 15 minutes at room temperature. We recommend that a swing-out bucket rotor should be used to minimize remixing of the plasma and platelets, particularly with plasma removal. While it is crucial that an essentially platelet-free sample be obtained if the specimen will be frozen for subsequent testing, APTT, PT/INR, and TT performed on fresh plasma samples are not affected by platelet counts of at least up to 200 x 10^9/L (200,000/µL). Samples that have visible hemolysis should not be used because of possible clotting factor activation and end point measurement interference. Some current instruments using an optical detector may have problems with end point determinations on samples that are icteric, lipemic, or contain substances that interfere with light transmission. Alternative methods (e.g., mechanical/electromechanical) should be considered.

The reliability of the centrifugation procedure should be validated every six months or after modification of the centrifuge to ensure plasma platelet counts are within acceptable limits.

5.2 Storage

The allowable time interval between collection of the specimen and testing of the sample will depend on the temperature encountered during transport and storage of specimen. Specimens for coagulation testing should be processed and stored as follows:

- Specimens for PT assays uncentrifuged or centrifuged with plasma remaining on top of the cells in an unopened tube kept at 18 to 24 °C should be tested within 24 hours from time of specimen collection. Storage at 2 to 4 °C may result in cold activation of Factor VII and therefore alter PT results. If the patient is on both heparin and a coumarin-based oral anticoagulant therapy, the PT may vary with time of storage of the specimen unless the PT reagent contains a heparin neutralizer.

- Specimens for routine APTT assays on nonheparinized patients uncentrifuged or centrifuged with plasma remaining on top of the cells in an unopened tube kept at 2 to 4 °C or 18 to 24 °C should be tested within four hours from time of specimen collection.

- Specimens for APTT assays suspected to contain unfractionated heparin kept at 2 to 4 °C or 18 to 24 °C should be centrifuged within one hour of collection and the plasma tested within four hours from time of specimen collection. If agitation of the specimen is likely after centrifugation, such as
transportation to a remote testing site, the plasma should be removed within one hour of collection and tested within four hours from the time of specimen collection.

- Specimens for other assays (e.g. thrombin time, protein C, Factor V, and Factor VIII) kept at 2 to 4 °C or 18 to 24 °C, should be centrifuged and tested within four hours from time of specimen collection.

If the testing is not completed within 24 hours for PT specimens and four hours for APTT and other assay specimens, plasma should be removed from the cells and frozen at -20 °C for up to two weeks or -70 °C for up to six months. A frost-free freezer should not be used. Frozen plasma specimens should be rapidly thawed at 37 °C while gently mixing and tested immediately; if testing cannot be performed immediately, the specimen may be held for a maximum of two hours at 4 °C until tested. The APTT may be affected on specimens that have been frozen.

6 Performance of Coagulation Assays

These are general guidelines applicable to most coagulation assays. The procedures for specific assays are covered in separate NCCLS documents.

6.1 Quality Control

The laboratory should follow generally accepted quality control practices. (For additional information, please see the current edition of NCCLS document C24—Statistical Quality Control for Quantitative Measurements: Principles and Definitions.) Specifically, laboratory personnel with appropriate experience should inspect the quality control results daily to evaluate for trends or shifts, as well as out-of-limit results. Individual patient values should be reviewed to look for unusual or unlikely patterns that can indicate a system malfunction or clerical errors. Maintenance of all instruments should be carried out in accordance with manufacturers’ directions and all actions documented. Manufacturers’ instructions for reagents and equipment should be followed. In addition, there should be periodic review (generally monthly) of quality control data to look for long-term changes in the analytic systems and, when appropriate, for the comparison of the laboratory’s results with those of a peer group.

Each laboratory should enroll in a proficiency-testing program acceptable to the relevant inspecting and accrediting agencies.

The laboratory should keep accurate and complete records of the lot numbers of reagents, reference materials, and, where possible, blood collection devices (if used). All reagents and solutions should be marked as to expiration date, date received, and reconstitution date (when applicable).

If trends, shifts, or out-of-limit values of quality control results are noted, or if spurious patient results are evident, an examination of the “entire coagulation test system” should be investigated to determine if the underlying problem is due to the phlebotomy procedure employed, the technologist(s) performing the testing, the reagent and instrument systems instituted, or some other “unexpected component” of the testing system.

6.2 Controls

Normal and abnormal controls should be run in accordance with the recommendations provided in the NCCLS guidelines for specific coagulation assays such as for PT and APTT assays (H47—One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test) and fibrinogen assay (H30—Procedure for the Determination of Fibrinogen in Plasma). Controls, once thawed or reconstituted, should not be refrozen or reused unless otherwise stated by the manufacturer. If the test values for the control samples are not within the established limits, appropriate action must be taken.
This can include testing new controls and method troubleshooting. Acceptable results must be obtained before patient samples are tested and reported.

6.3 End Points

End point measurements are read by a variety of optical or electromechanical methods using manual, semiautomated, or automated devices. Manual procedures are difficult to standardize and not widely used.

6.4 Reference Intervals

Reference intervals using normal populations of appropriate age and as otherwise defined should be established by each laboratory, and they should be verified with any change in reagent lot number, instrument, collection system, or at least once a year. For more information on reference intervals, see the most current edition of NCCLS document C28—How to Define and Determine Reference Intervals in the Clinical Laboratory.

6.5 Single vs. Duplicate Determinations

Determinations are commonly performed in duplicate, and the mean of the two values is reported. With improvements in the performance of semiautomated and automated coagulation instruments, singlet testing is acceptable, if appropriate quality standards are met. Single determinations should only be considered with the use of automated equipment.

7 Reporting of Results

Reporting systems are addressed in each specific assay guideline such as for PT and APTT assays (NCCLS document H47—One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test), and fibrinogen assay (NCCLS document H30—Procedure for the Determination of Fibrinogen in Plasma).
References


Select the curve for the total volume of anticoagulated blood required (10, 5, 2, or 1 mL). Enter the chart at the patient's packed cell volume on the horizontal axis and read off the corresponding volume of anticoagulant solution on the vertical axis. Place the volume in a collection tube and add blood up to the required total volume. The chart assumes a normal packed cell volume of 43%; in that case, 1 volume of anticoagulant is made up to 10 volumes with blood.

Alternatively, the anticoagulant volume may be calculated from the expression:

\[
x = \frac{(100 - \text{PCV})}{(595 - \text{PCV})} \text{vol},
\]

where \(x\) is the volume of anticoagulant required to prepare unit volume of anticoagulated blood, and PCV is the packed cell volume in %. e.g., to determine the volume required for 5 mL anticoagulated blood, calculate 5 \(x\) mL.
Summary of Comments and Subcommittee Responses


General

1. NCCLS has inconsistencies in their own publications (H3 and H21) when it comes to the order of draw for coagulation testing relative to tissue thromboplastin contamination and procurement of a “discard tube.” In performing intralaboratory studies at our institution and sister institutions, results showed no significant clinical or statistical significance on any of the assays between each of the specimen tubes performed at each individual laboratory. Also, our results indicated that NCCLS guidelines for obtaining a second tube when performing coagulation testing should be eliminated when the revised document is published.

- The text in Section 4.1 has been revised to reflect the recommendations in the NCCLS document H3-A5 regarding the use of discard tubes during specimen collection for coagulation testing.

2. Please provide documentation to support the recommendation that fibrinogen determinations be performed within four hours of collection. It is not unusual for 100 veterinary specimens to arrive at our laboratory simultaneously for PT, APTT, and fibrinogen assays. Using two MLA 1000 Cs, it is still very difficult to perform all these tests within four hours. Many are of the opinion that fibrinogen is not that labile.

- The subcommittee is not aware of any studies or documentation that fibrinogen stability is greater than four hours.

Section 4.2.3, Citrate Concentration Adjustments (Formerly Section 5.2.3)

3. Has the correction factor for hematocrits above 55% changed with the advent of the 2.7 mL plastic tube? What about correcting for hematocrits below 20%?

- The correction factor should not change based on the size of the tube. The blood-to-anticoagulant ratio should be the same no matter the size of the tube. The subcommittee is not aware of any data available related to correction for hematocrits below 20%.

Section 5.1, Preparation of Suitable Plasma Specimens (Formerly Section 6.1)

4. We have not found supporting documentation for the recommendation that platelet counts of <10 x 10^9/L be present in coagulation studies. By conducting a study of the significance of platelet counts in coagulation studies, we have, however, determined that platelet counts of at least 200 x 10^9/L can be present in plasmas without compromising the accuracy of the PT, INR, or diagnostic PTT determinations (Carroll WE, Wollitzer AO, Harris L, et al. The significance of platelet counts in coagulation studies. J Med. 2001;32:83-96). This information should be taken into account during the next revision of the document.

- The subcommittee is aware that studies suggest platelet counts of >10 x 10^9/L are appropriate for routine coagulation testing. However, it is not always known for what testing the specimen will be used. Therefore, the text in this section has been revised to recommend platelet counts >10 x 10^9 are acceptable for routine APTT, PT/INR, and TT testing if tested on fresh specimens. Specimens that will be frozen for subsequent testing should be essentially platelet free. This document provides guidelines for the general performance of coagulation assays, and platelet counts >10 x 10^9/L are not acceptable for all testing (e.g., lupus anticoagulants, other phospholipid antibodies, and heparin monitoring).

Section 5.2, Storage (Formerly Section 6.2)

5. The text of this section indicates that specimens for PT assays can be uncentrifuged, or centrifuged, with plasma remaining on top of the cells in an unopened tube at 2 to 4 °C for up to 24 hours and used for testing. If testing cannot be completed within this time frame, the plasma may be removed and stored frozen, at -20 °C or -70 °C. It does not address the situation in which samples have been collected, centrifuged, plasma removed, and stored at 4 °C. Does the stability time become four hours under these conditions?

- The subcommittee is not aware of any studies or documentation addressing these specific conditions; therefore, a recommendation was not included.
Summary of Delegate Comments and Working Group Responses

H21-A4: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition

General

1. In the Subcommittee Comments and Questions page, question 2 does not appear to have been answered. The writer asks for documentation to substantiate the four-hour requirement.

- The working group is unaware of published studies; therefore, the conservative recommendations were presented. The recommendations for handling fibrinogen specimens parallel the APTT times. If laboratory personnel believe that four hours is too short, they should perform validation studies for the use within their laboratory.

2. The document is very elementary and actually is of some, but not much, help to someone who needs advice on collection of blood for coagulation testing. What is generally missing is suitable advice regarding the problems that can be associated with a sample that is not “ideal,” but may be the only sample available.

- The working group believes it has addressed the major aspects of the majority of samples received in the laboratory. Unfortunately, the document cannot address every contingency. The working group believes that if the sample falls outside the established parameters, then the sample should not be tested, or tested and interpreted with caution.

3. There is no discussion of the ACT (a whole blood version of the aPTT, but modified by omitting phospholipids), a test that is used for monitoring unfractionated heparin in the OR when patients are on cardiopulmonary bypass. It might be argued that this is outside the scope of this document, but if the document is to be used by individuals who need instructions on collecting blood for coagulation testing, mentioning this could prevent some confusion. A similar ambiguity exists for the prothrombin time and prothrombin time test. Again, there is error here.

- The ACT is addressed in NCCLS document H49—Point-of-Care Monitoring of Anticoagulation Therapy.

Section 3, Definitions

4. Anticoagulant – “or blood products.” This is a bit strange because it is either irrelevant, or needs to be expanded if the intent is to indicate that testing blood products may be confounded by the presence of an anticoagulant in them. Here, some examples and where the particular anticoagulant is used could be helpful.

- The definition has been revised to delete the phrase “or blood products.”

5. Coagulation factors - the listing of Factor III and Factor IV for thromboplastin and calcium as the first entry (OK, consistency), but these designations are not used. If consistency is the goal, then a note that indicates that the terms in common use are thromboplastin and calcium needs to be present.

- The phrase “commonly termed” has been added to Factor III and Factor IV as suggested.

6. There are inconsistencies in the two definitions for “time” and for “time test.” There might be a desire to distinguish between the time and the test that is based on the measured time interval, but the presentation does not do this very well. An example of the inconsistency is: Activated partial thromboplastin time, APT (probably a typo here, aPTT or APTT) is indicated as measuring the intrinsic coagulation pathway, but the activated partial thromboplastin time test is indicated as measuring the intrinsic and common coagulation pathway... No, both are indicators (loosely) of the intrinsic and common pathways. The test (APTT) is used for monitoring heparin, as indicated, but not low molecular weight heparins - the weakness is that there is no qualification.

- The working group believes the definitions are correct and understandable to differentiate the distinction of test vs. time. However, in response to the comment, the text has been revised to include “intrinsic and common pathways” in the definition of activated partial thromboplastin time. The parenthetical statement, “(but not low molecular weight heparins),” has been added to the activated partial thromboplastin time test definition.

7. Under PT/(Time of tissue factor-induced coagulation), calcium chloride is used as an example of a thromboplastin reagent. No!

- In response to the comment, the text has been revised as follows: “(e.g., tissue factor and calcium chloride).”
8. The thrombin time and TT test have similar problems, but there is also a distinction that needs to be made regarding the thrombin used. Some grades of thrombin are unacceptable; they are proteolytically degraded. The suitable material is alpha thrombin. Some information needs to be present to advise or warn the individuals that some thrombins and stored thrombin preparations may be unsuitable. Again, it could be argued that this should be dealt with elsewhere, but a reference or some other information should be available.

- The definition of thrombin time has been revised to include “suitable” before the statement, “thrombin has been added.” The reader has also been referred to the most current edition of NCCLS document H30—Procedure for the Determination of Fibrinogen in Plasma, for additional information.

9. Line one should read aPTT, not APT.

- The typographical error has been corrected.

10. Coagulation factors: Following the word “Fitzgerald” should be “factor.”

- The term “factor” has been added after Fitzgerald.

11. Dead space volume: is also the volume that cannot be aspirated from vial.

- This is correct, but we are discussing catheter dead space in this section.

12. In the definition for Prothrombin time (PT)—“e.g., calcium chloride” should be “e.g., tissue factor and calcium chloride.”

- The text has been revised as suggested.

Section 4.1, Methods for Obtaining Blood Specimens

13. A question was raised regarding two-tube collection, specifically regarding the use of a coagulation tube for the first tube. The concern is that this is a setup for error; two indistinguishable tubes, the first one being unsuitable but mixed up because of no distinction. Another question was raised regarding inverting the tube at least four times without any statement about possible excessive inversions.

- The working group agrees that a potential mix-up of a first tube and second tube can occur. However, if the first tube is a coagulation tube, then it should be marked as such or, better yet, the second tube should have the label on before drawing. Otherwise, the working group recommends a nonadditive tube be used.

The following statement has been added to the end of the last paragraph in this section: “Excessive mixing can cause hemolysis and/or platelet activation, leading to erroneous results.”

Section 4.2.3, Citrate Concentration Adjustments

14. Following “0.55” should be “L/L.”

- The document has been revised as suggested.

Section 4.3, Needle Gauge

15. One person questioned the range of needle sizes, particularly the small diameter, 22- and 23-gauge needles. Some problems in platelet assays have been encountered with the small-gauge needles. A warning is suggested.

- The working group is unaware of published studies related to small-gauge needles causing platelet activation or hemolysis. Therefore, the text has been revised to encourage the phlebotomist to assess the subject prior to performing the phlebotomy. This assessment should minimize the preanalytical variables that can cause hemolysis and thrombogenicity.

Section 5.1, Preparation of Suitable Plasma Specimens

16. As I see it, there would no longer be a requirement for refrigerated centrifugation for these tests, but the document does not state that specifically.

- The working group found that either room temperature or refrigerated centrifugation will work, so did not recommend too strongly for either one.
17. Text lines 5 and 10 – µL is the unit most recognized for reporting platelet counts.

- The standard reporting of a platelet count is x10⁹/L.

18. I suggest additional information that could be added to Section 5.1, “Preparation of Suitable Plasma Specimens,” providing a guiding statement regarding questionable sample suitability when collection tubes are overfilled for the quantity of citrate anticoagulant in the collection tube.

- Data suggest that an overfilled standard vacuum tube will not give erroneous results until it is overfilled to more than 120%. This is not a usual problem.

19. I have questions on the validation of the centrifugation process every six months and after major repairs and adjustments to centrifuge.

- It is important to verify the platelet count every six months or after modification of the centrifuge. These circumstances can cause erroneous results in some samples, especially stored. Therefore, validation of the centrifugation process must be performed.

20. There is a publication regarding the platelet count limits, Brit J Haematol. 2003;120:825-828, that addresses problems in some APTT assays, specifically when an APC resistance test is to be performed.

- The suggested reference has been added at the end of the third sentence in the first paragraph.

Section 5.2, Storage

21. Recommendations for transport and storage of PT specimens apply to outpatient samples used for monitoring of oral anticoagulant therapy. Twenty-four hours would seem excessive for specimens from patients with overt or non-overt DIC or patients with moderate to severe liver disease. In these cases, the recommendations made for the aPTT may be more applicable. In addition, we have never found storage at 2 to 4 °C to be a practical issue in the clinical performance of the PT.

- The working group believes that the majority of PT samples are for oral anticoagulant therapy monitoring. It certainly is prudent clinically to test DIC patients sooner (within four hours), but PT samples are stable for up to 24 hours. The working group did not recommend 2 to 4 °C, as it causes cold activation and may shorten the PT values. Therefore, PT samples should be kept at 18 to 24 °C or frozen.

22. How does cold (2 to 4 °C) affect PT?

- In some samples, Factor VII is activated by the cold and the PT can shorten. See response to Comment 21.

23. The last paragraph of Section 5.2 recommends plasma for APTT testing should be separated and frozen, if not tested in four hours. The last sentence of the paragraph then states: “The APTT may be affected on specimens that have been frozen.” Freezing affects APTT…how?

- In some samples, Factor IX can be activated, causing a shortening of the APTT.

24. What does this document recommend about temperature control and short-term sample storage of plasma?

- The temperature should be consistent during short- or long-term storage. Short-term storage of plasma can be at room temperature for up to four hours for APTT and 24 hours for PT. Otherwise, the plasma sample should be frozen.

25. Specimens for routine APTT assays on nonheparinized...kept at 2 to 4 °C. No - cold activation occurs (a difference in the temperature dependence of rate constants for activation and inhibition that results in net activation in the cold.) There is a Factor XIIa test that this would generate artifactually high results. For the various other recommendations in this category, two reviewers asked - evidence? References?

- Cold activation does not occur within four hours at 2 to 4 °C. It is unknown as to what effect cold has on Factor XIIa levels. Reference 15 provides the data for the basis of this recommendation.
Section 6.1, Quality Control

26. Why not refer to NCCLS document C24 for quality control?

- The reader has been referred to NCCLS document C24—Statistical Quality Control for Quantitative Measurements: Principles and Definitions at the end of the first sentence in this section.

Section 6.4, Reference Intervals

27. Current regulations regarding sample collection probably preclude this approach. This is now wrong advice.

- The working group agrees that the new HIPAA rules make it harder to do research in clinical laboratory coagulation, but does not limit the use of normal plasmas for internal determination of reference ranges, etc.

Appendix

28. Percentages expressed as SI units should be followed by “L/L.”

- The percentages expressed in this Appendix are in whole numbers, not in SI format (i.e., with a decimal) as in Section 4.2.3.
The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; 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 HS1—*A Quality System Model for Health Care*. The quality system approach applies a core set of “quality system essentials (QSEs),” basic to any organization, to all operations in any healthcare service’s path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The quality system essentials (QSEs) are:

- Documents & Records
- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Information Management
- Process Control
- Occurrence Management
- Assessment
- Process Improvement
- Service & Satisfaction
- Facilities & Safety

H21-A4 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

H21-A4 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Adapted from NCCLS document HS1—*A Quality System Model for Health Care*.
Related NCCLS Publications*


H1-A5 Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003). This document contains requirements for venous blood collection tubes and additives, including technical descriptions of ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin compounds used in blood collection devices.

H3-A5 Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003). This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

H30-A2 Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline—Second Edition (2001). This document provides general guidelines for performing the fibrinogen assay in the clinical laboratory. It also includes reporting of results and in vivo and in vitro conditions that may alter results.

H47-A One-Stage Prothrombin Time Test (PT) and Activated Partial Thromboplastin Time Test (APTT); Approved Guideline (1996). This document provides guidelines for performing the PT and APTT tests, for reporting results, and for identifying sources of error.

M29-A2 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2002). Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions by preventing the laboratory 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.
NOTES