7 Considerations in Performing the PT and APTT Tests

7.1 Manufacturers' Instructions

Follow the manufacturers' instructions for reagents and equipment.

7.2 Acceptable Variability

Analytical error (see Section 10.4) is influenced by the reagents, instruments, sample delivery devices, and timer, resulting in imprecision. The total day-to-day coefficient of variation (CV) of the analytic system should be less than 5% with the same lot of normal and abnormal control plasmas.

7.3 Reagent Grade Water

Use Type I reagent grade water, as specified in NCCLS document C3-A2, *Preparation and Testing of Reagent Water in the Clinical Laboratory–Second Edition; Approved Guide-line*, or as otherwise specified by the manu-facturer. If the laboratory uses a different type of water, it should document its acceptability.

7.4 Calcium Ion Concentration

Use the concentration of calcium ions recommended by the manufacturer of the PT and APTT reagents.

7.5 Conditions of the Test System

Use only clean collection tubes, storage tubes, plastic ware, and delivery systems in the performance of the tests.

7.6 Controls Outside of Stated Limits

If the test values for the control samples are not within the stated limits, check reagents, control plasma, and equipment. Document the identifiable causes and actions undertaken to identify and correct the problem before any patient plasma data are reported.

7.7 Control Plasma Collection, Handling, and Storage

If control plasma samples are prepared within the laboratory, they must be prepared and stored according to acceptable methods. Collect blood used for preparation of control plasmas into citrate anticoagulant. The citrate solution and ratio of citrate to blood volume should be identical to that used in the collection of test specimens. Handle and store control plasma(s) under conditions identical to, or as similar as possible to, those used for storage of test samples. See NCCLS document H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays—Second Edition; Approved Guideline* for more information on coagulation specimen collection, handling, and storage.

7.8 Frequency of Control Testing

Test controls at the initiation of testing each day and at least once each shift, or with each group of assays. Controls should also be tested with each reagent change or major instrument adjustment. In laboratories where there is a heavy workload of PTs and/or APTTs, test a normal and an abnormal control at a minimum of every 40 samples.

7.9 Reproducibility of Duplicates

The size of the difference between duplicate measurements is commonly used as a criterion for result acceptability. This is helpful as a check on system imprecision and/or sporadic analytic errors. Although the exact size of difference that constitutes the appropriate operational limit may vary with the analytic system used, the difference between duplicate results should agree within 10% of their mean value.

7.10 Reference Intervals

A reference interval should be established by each laboratory and it 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 NCCLS document C28-A, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory; Approved Guideline*.

7.11 General Quality Control

The laboratory should follow generally accepted quality control practices. 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. 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 results with those of a peer group.

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

The laboratory should keep accurate and complete records of the lot numbers of reagents, reference materials, and, where possible, evacuated tubes (if used).

8 Performance of the Prothrombin Time (PT) Test

8.1 PT Principle

Thromboplastin and a source of calcium ions are combined with test plasma at 37 °C; the PT is the time, in seconds, required for a detectable fibrin clot to form.

8.2 PT Reagents: Thromboplastins

Usually, the thromboplastin reagent is a buffered thromboplastin–calcium mixture supplied by the manufacturer. There is great variability in ISI values in the responsiveness of different PT reagents.

8.3 PT Performance Temperature

Perform the test at 37 ± 1 °C. Prewarm aliquots of plasma to 37 °C for no more than 10 minutes before performing the test. Follow the manufacturers' instructions that describe preparation and handling of individual thromboplastin reagent.

8.4 PT Test Procedure

Initiate the PT by mixing two parts of prewarmed thromboplastin–calcium reagent and one part of prewarmed citrated plasma. Start a timing device the instant the reagents are mixed. Record the time required for clot formation.

8.5 PT End Point

Measure the end point by a variety of optical or electromechanical methods using manual, semi-automated, or automated devices. Determinations are commonly performed in duplicate and the mean of the two values is reported. With improvements in the precision of semi-automated and automated coagulation instruments, singlet testing is acceptable, if appropriate quality standards are met.[17] For more information on single versus duplicate determinations, refer to NCCLS document H21-A2.

8.6 Reporting PT Results

The laboratory should report the results of the PT test to the nearest half of a second or less along with the normal reference interval and the INR. The ratio of the PT to the geometric mean of the reference interval may also be reported. It has been shown that for patients stabilized on oral anticoagulant therapy, the reporting of an INR is preferred because it reduces intermethod variability.[2,4,5,15,16,18] Conversion to an INR in effect calibrates the results of a particular reagent/instrument system to results of an international reference reagent. Despite this, INR values produced by different test systems can still vary considerably.[19] This variability is diminished by universal use of highly responsive thromboplastin reagents, with an ISI below 1.5.

9 Performance of the APTT Test

9.1 APTT Principle

Citrated test plasma, a contact activator, and procoagulant phospholipids are mixed and incubated at 37 °C. The contact agent acti-

vates Factor XI and Factor XII. The phospholipid provides a surface for interaction of coagulation factors. After incubation, an appropriate concentration of calcium ions is added, and time to clot formation is measured. Calcium ions promote activation of the intrinsic coagulation cascade subsequent to Factor XIa.

9.2 APTT Reagent

The APTT reagent is a mixture of partial thromboplastin and contact factor activator. The activator may be celite, kaolin, silica, ellagic acid or other suitable substances. The APTT reagent/instrument combination should be able to detect abnormally prolonged results with plasmas that have less than 0.3 U/mL (30% factor activity) of the following coagulation factors: VIII, IX, XI.

9.3 APTT Performance Temperature

Perform the test at 37 ± 1 °C.

Prewarm aliquots of plasma at 37 °C for no more than 10 minutes before performing the test. Follow the manufacturers' instructions that describe preparation and handling of individual APTT reagents.

9.4 Contact Activation Time

The contact activation time refers to the duration of incubation of test plasma and APTT reagent. Rigid standardization of contact activation time is important. Because this varies with the instrument and particular APTT reagent used, follow the manufacturer's instructions. For manual procedures, use a stopwatch or a similarly accurate timing device.

9.5 APTT Test Procedure

The APTT is a two-stage test. Initiate the first stage by mixing one part APTT reagent (see Section 9.2) and one part citrated plasma. Simultaneous with the mixing of the reagent and the plasma, start a timing device to measure the exact contact activation time. At the end of the recommended activation time, initiate the second stage by adding one part of prewarmed calcium chloride (see Section 7.4), and simultaneously starting a timer. Record the time required for clot formation.

9.6 APTT End Point

The end point is the formation of a fibrin clot. It can be measured by a variety of optical or electromechanical methods using manual, semi-automated, or automated devices. Determinations are commonly performed in duplicate and the mean of the two values is reported. With improvements in the precision of semi-automated and automated coagulation instruments, singlet testing is acceptable, if appropriate quality standards are met.[17] For more information on single versus duplicate determinations, refer to NCCLS document H21-A2.

9.7 Heparin Sensitivity[20]

Because the APTT is commonly used for monitoring heparin therapy, the APTT reagent/instrument system should be adequate-ly responsive to standard heparin. The thera-peutic APTT range for heparin therapy should be determined in each hospital laboratory by establishing the APTT range corresponding to a recommended heparin concentration range, preferably using plasma from patients on heparin therapy (ex vivo). This requires the availability of an assay for measuring heparin concentration (e.g., protamine sulfate titration, anti Xa chromogenic assay). The therapeutic APTT range determined using plasmas from normal subjects spiked in vitro with known heparin concentrations is higher generally, but it is also acceptable.

9.8 Lupus Anticoagulants[21]

APTT reagents are variably sensitive or insensitive to lupus anticoagulants. The reagent manufacturer should provide sufficient documentation with respect to lupus anticoagulant sensitivity. Also, national proficiency testing programs provide appropriate additional information about differences in reagent sensitivity to lupus anticoagulants. Note, however, that there is considerable heterogeneity among individual pa-

tients; consequently, no single reagent will detect all lupus anticoagulants.

9.9 Reporting of APTT Results

The laboratory should report the results of the APTT test to the nearest second or less along with the reference interval.

10 Sources of Error

10.1 Specimen- or Sample-Related Problems

Following are several specimen- or sample-related problems:

- Overfill or underfill of collection tubes
- Failure to correct the citrate volume for persons with high (>0.55) packed cell volume (PCV; hematocrit) (See NCCLS document H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays–Second Edition; Approved Guideline*)
- Incorrect volume, type (e.g., EDTA or oxalate), or concentration of anticoagulant in the collection tube
- Clotted, hemolyzed, icteric, or lipemic specimens (refer to NCCLS document H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays–Second Edition; Approved Guideline*, for more information)
- Inadequate or too vigorous mixing of the specimen with the reagents
- Contaminated collection or storage tubes
- Contamination with heparin
- Improper or defective specimen collection tubes.

10.2 Reagent-Related Problems

Following are several reagent-related problems:

- Contaminated reagents
- Reconstitution with incorrect diluent volume
- Reconstitution with other than the recommended diluent
- Defects in the reagent due to mishandling in shipping or storage
- Use of the reagent beyond the stated reconstituted stability date or beyond the expiration date.

10.3 Other Pre-Analytical Errors

Pre-analytical errors include delay in or use of nonstandardized procedures for transporting, processing, storing, or testing the specimen.[16] (See NCCLS document H21-A2, *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays– Second Edition; Approved Guideline*, for more information.)

10.4 Analytical Errors

Analytical errors (see Section 7.2) can be due to the following circumstances:

- Incorrect incubation time or activation time
- Inaccurate or imprecise dispensing of reagents
- Failure to use proper instrument operating procedures
- Instrument malfunction, such as defective bulb, incorrect temperature, reagent splash, poor reagent delivery, or electrical interferences.