Mixing Study, Incubated APTT

Background Information

The activated partial thromboplastin time (APTT) is one of the most commonly used screening tests to investigate bleeding patients, monitor anticoagulant therapy and as a screening test prior to surgery. The APTT measures the integrity of the intrinsic and common pathways of the coagulation cascade. The prothrombin time (PT), another common screening test, measures the integrity of the extrinsic and common coagulation pathway. The APTT is measured as the number of seconds for the patient's plasma to form a fibrin clot after the addition of an intrinsic pathway activator, phospholipid and calcium. A prolonged APTT can be caused by a coagulation factor deficiency or the presence of an inhibitor. The mixing study, incubated APTT, is used to investigate the cause of a prolonged APTT result. The mixing study is performed by measuring the APTT in the patient's plasma, then mixing an equal volume of the patient's plasma and normal pooled plasma (NPP) and repeating the APTT tests immediately and after one-hour incubation. The components of the panel include PT screen, APTT screen, APTT Immediate Mix and APTT Incubated Mix, as well as a thrombin time and heparin anti-Xa assay, if needed.

The principle of the mixing study can be summarized as:

1. If the prolonged APTT screen is due to a factor deficiency, mixing with an equal volume of NPP, which has approximately 100% of all coagulation factors, will replace the patient's deficient factor. This result in an APTT immediate mix is shortened or corrected into the reference range.
2. If the prolonged APTT screen is due to the presence of an inhibitor, mixing with an equal volume of NPP will not shorten or correct the prolongation of APTT in repeated tests. The reason is the inhibitor in the patient's plasma is present in excess and binds to coagulation factors or protein/phospholipid complexes in both the patient's plasma and NPP.

Correction of the APTT in the mixing study suggests a coagulation factor deficiency in either the intrinsic pathway (factors VIII, IX, XI and XII, high-molecular-weight kininogen [HMWK] or prekallikrein [PK]), or in the common pathway (also prolonged PT) such as factor II, V and X. Deficiency of factors VIII, IX, and XI will present with bleeding, however, deficiency of factor XII, or prekallikrein will not increase bleeding risk, but may increase thrombotic risk. Further testing, such as clotting factor assays, is necessary to diagnose a specific factor deficiency. See Figure 1 for the diagnostic algorithm used in the laboratory.

There are three different types of inhibitors:

1. Inhibitors directly against specific factors such as factor VIII or factor V inhibitors,
2. Anticoagulants such as heparins, fondaparinux, dabigatran and other direct thrombin inhibitors, and
3. Non-specific inhibitors such as lupus anticoagulants.

Some inhibitors will demonstrate a delayed type inhibitor pattern, with time and/or temperature dependence. In cases with a delayed type inhibitor, the APTT Immediate Mix will correct to within the reference range, however, the APTT Incubated Mix will be prolonged. Although rare, the presence of a factor inhibitor, such as a factor VIII inhibitor, will increase the risk of life-threatening bleeding. The presence of a factor inhibitor can be confirmed by a Bethesda assay for that factor.

The presence of heparins, fondaparinux, dabigatran or other direct thrombin inhibitors can cause prolongation of both the APTT Immediate Mix and APTT Incubated Mix. Careful clinical and medication history, and additional thrombin time with heparin assay (anti-Xa inhibition assay) can exclude the presence of anticoagulants.

The presence of lupus anticoagulants, which are antibodies against protein-phospholipid complexes, will increase the

risk of thromboembolism. The presence of low level non-specific inhibitors in the patient's plasma may demonstrate a prolonged APTT Incubated Mix similar to a delayed type inhibitor. If the clinical history suggests a lupus anticoagulant, further testing including phospholipid based screening tests, phospholipid dependency assays, and exclusion of the presence of inhibitors, in addition to mixing study, incubated APTT, is necessary (see diagnostic algorithm for lupus anticoagulant).

The adequate performance of the mixing test and accurate interpretation is important because the presence of a specific factor inhibitor, non-specific inhibitor such as lupus anticoagulant, anticoagulants or factor deficiency have different clinical manifestations and require different clinical management.

Clinical Indications

The mixing test, incubated APTT, is indicated when the cause of a prolonged APTT result needs to be investigated.

Interpretation

1. If the APTT Screen is prolonged with a normal APTT Immediate Mix and APTT Incubated mix, this indicates a factor deficiency in the intrinsic or final common pathway. If the PT is normal, this suggests an intrinsic pathway deficiency (VIII, IX, XI, XII, PK, HMWK). If the PT is prolonged, this suggests a common pathway deficiency (fibrinogen, II, V, X).
2. If the APTT Screen is prolonged, with a normal APTT Immediate Mix, but an abnormal APTT Incubated Mix, this indicates the presence of a delayed inhibitor such as specific factor inhibitors, most commonly factor VIII inhibitor, and small numbers of lupus anticoagulant.
3. If the APTT Screen is prolonged, with an abnormal APTT Immediate Mix and abnormal APTT Incubated Mix, this favors a non-specific inhibitor such as a lupus anticoagulant, and anticoagulants such as heparin, fondaparinux, dabigatran or other direct thrombin inhibitors.

Methodology

The PT Screen is performed using Innovin® (Dade Behring, Inc.) reagent and STAR Evolution® Analyzer (Diagnostica Stago, Inc.). The PT Screen is included to localize abnormalities to common, intrinsic and extrinsic pathway. The APTT Screen is performed using the PTT-Automate reagent and STAR Evolution® analyzer (both Diagnostica Stago, Inc). The mixing studies are performed by mixing the patient's plasma with an equal volume of the NPP (Cryocheck; Precision Biologic, Inc). For the APTT Immediate Mix, the APTT is performed immediately after mixing the plasmas. For the APTT Incubated Mix, the APTT is performed after one-hour incubation at 37ºC. The NPP serves as a negative control; two levels of positive control are performed; lupus positive plasma (Precision Biologic, Inc) and weak lupus positive plasma (Precision Biologic, Inc). The thrombin time (Diagnostica Stago, Inc) will be measured in specimens with prolonged APTT. If the TT is prolonged, a heparin assay (anti-Xa inhibition assay; Rotachrom Heparin kit, Diagnostica Stago, Inc.) by a chromogenic assay will be performed to distinguish a heparin effect from a direct thrombin inhibitor.

Specimen Collection and Handling

Discontinue coumadin therapy for 14 days; direct thrombin inhibitors, fondaparinux and heparin 2 days prior to collection. The presence of heparin, fondaparinux, dabigatron or other direct thrombin inhibitor in the specimen may interfere with test results. Blood should be collected by routine venipuncture in a 3.5mL light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5mL with an appropriate ratio of anticoagulant is acceptable. Specimens improperly collected, stored, misidentified or of insufficient volume are unacceptable.

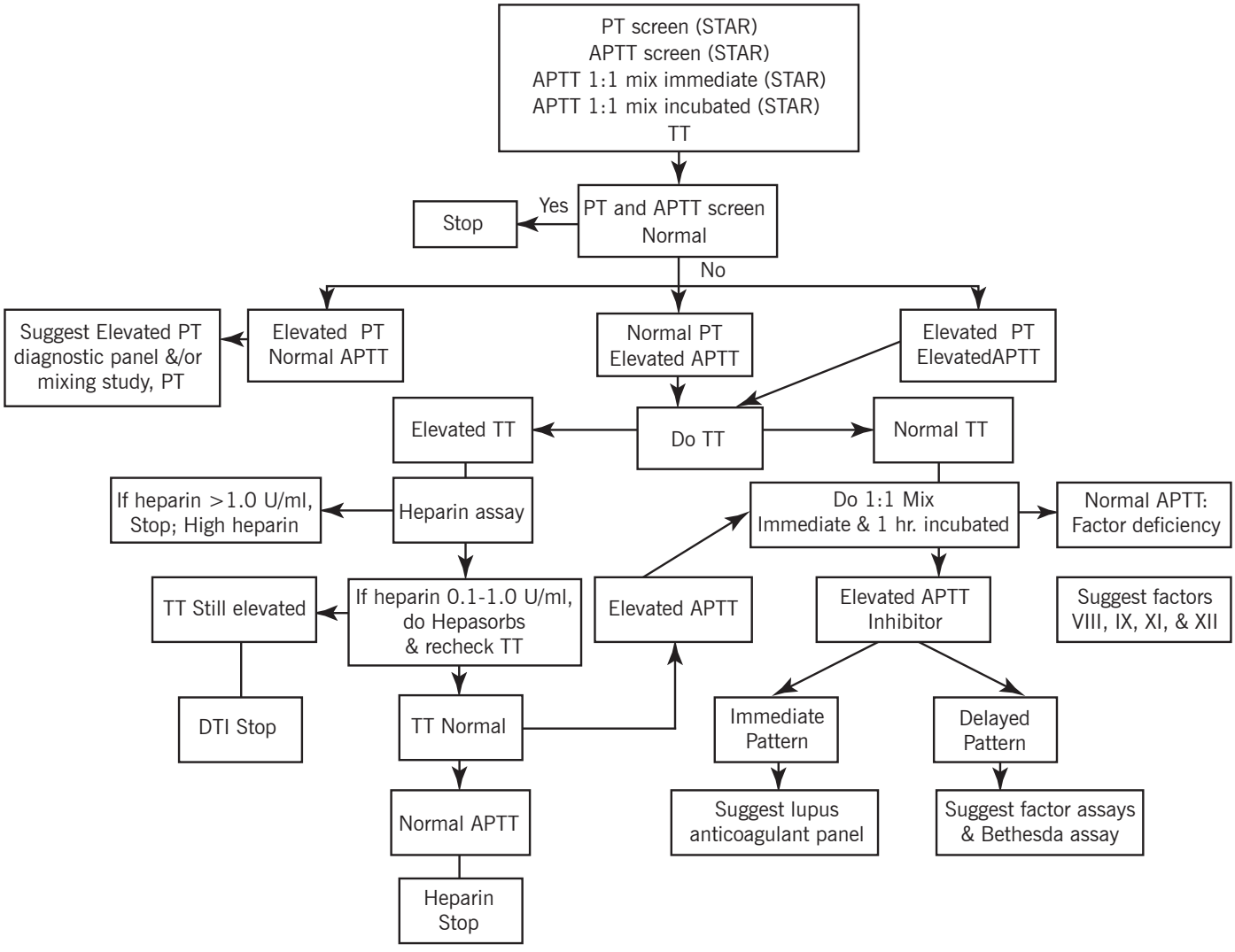
Suggested Reading

1. Kottke-Marchant K. An Algorithmic Approach to Hemostasis Testing. CAP Press (2008).
2. Devreese KMJ. Interpretation of normal plasma mixing studies in the laboratory diagnosis of lupus anticoagulants. Thrombosis Research, 2007;119(3):369-376.

3. Favaloro EJ, Bonar R, Duncan E, Earl G, Low J *et al*. Misidentification of factor inhibitors by diagnostic haemostasis laboratories recognition of pitfalls and elucidation of strategies. A follow up to a large multi-center evaluation. *Pathology.* 2007;39(5):504-511.

4. Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothormbin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clinic Proceedings. 2007;82(7):864-873.

Mixing Study, APTT Incubated



Abbreviations: PT – prothrombin time; **APTT** – activated partial thromboplastin time; **TT** – thrombin time; **DTI** – direct thrombin inhibitors

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Test Overview

Test Name	Mixing Study, Incubated APTT
Ordering Mnemonic	PTTIM
Test Included	PT Screen APTT Screen APTT Immediate Mix APTT Incubated Mix Thrombin Time & Heparin Assay Interpretation & Pathologist Review
Reference Range	See interpretation PT Screen: reference range; 8.4-13.0 seconds APTT Screen: reference range; less than 33.2 seconds APTT Immediate Mix; reference range; less than 33.2 seconds APTT Incubated Mix; reference range; less than 36.0 seconds TT: reference range; less than 18.6 seconds Heparin assay: reference range; less than 0.1 U/mL
Specimen Requirements	Testing Volume/Size: 2 mL; Type: Plasma; Tube/Container: Sodium citrate (lt. blue); Transport Temperature: Centrifuge, aliquot and freeze.
Specimen Collection and Handling	Discontinue coumadin therapy for 14 days; direct thrombin inhibitors and heparin 2 days prior to collection. The presence of heparin, fondaparinux, dabigatran or a direct thrombin inhibitor in the specimen may interfere with test results. Collection of blood by routine venipuncture in a 3.5mL light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5mL with an appropriate ratio of anticoagulant is acceptable. Specimens other than 3.2% trosodium citrate plasma and those that are improperly collected, stored, misidentified or of insufficient volume are unacceptable. Also refer to “Criteria for rejection and special handling of coagulation specimens.”
Test Ordering Information	3.2% sodium citrate is the preferred anticoagulant recommended by CLIS.
Billing Code	88605
CPT Codes	85610, 85730, 85732 (x2), 85670, 85520, 85390

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