

# Monitoring the Anti-Xa Anticoagulants, from Heparin to Eliquis

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## LEARNING OBJECTIVES

1. Describe the physiologic action of heparin, low molecular weight heparin, and pentasaccharide on antithrombin and activated coagulation factor X.
2. Prepare an ex vivo “Brill-Edwards curve” and employs the partial thromboplastin time to monitor unfractionated heparin.
3. Employ the prothrombinase-induced clotting time and the chromogenic anti-Xa assay to monitor unfractionated heparin, low molecular weight heparin, pentasaccharide, or direct anti-Xa therapy.

**ABBREVIATIONS:** AMI - acute myocardial infarction; APTT or PTT - activated partial thromboplastin time; AT - antithrombin; CAD - coronary artery disease; DTI - direct thrombin inhibitor; DVT - deep venous thrombosis; FDA - US Food and Drug Administration; GFR - glomerular filtration rate; HIT - heparin-induced thrombocytopenia with thrombosis; LMWH - low molecular weight heparin; PE - pulmonary embolism; PiCT - prothrombinase-induced clotting time; PT - prothrombin time; RI - reference interval; RUO - research use only; SERPIN - serine protease inhibitor; TAT - thrombin-antithrombin; UFH - unfractionated heparin; VTE - venous thromboembolism.

**INDEX TERMS:** Anticoagulants, heparin, fondaparinux, rivaroxaban, apixaban, activated partial thromboplastin time, anti-Xa heparin assay, thrombosis, thromboembolic disease, coronary artery disease.

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## Standard Unfractionated Heparin Therapy

Unfractionated heparin (UFH) is a crude mixture of sulfated linear *glycosaminoglycans* extracted from porcine mucosa composed of an average of 60 saccharide units.<sup>1</sup> UFH is one of nature's most negatively charged molecules with a molecular weight of 3000–30,000 Daltons and a median of 15,000 Daltons.<sup>2</sup> A pentasaccharide sequence that binds a specific site on plasma antithrombin (AT, antithrombin III, AT III) appears on one-third of UFH molecules. This sequence provides the catalytic anticoagulant action of UFH as it binds its AT receptor site. UFH-bound AT undergoes a conformational change, exposing a second site that covalently inactivates the coagulation pathway serine proteases IIa (thrombin), IXa, Xa, XIa, and XIIa. We take clinical interest only in AT binding of thrombin and Xa, despite its additional properties. Activated AT is a *serine protease inhibitor* (SERPIN), and the protease binding reaction yields an inactive plasma complex thrombin-antithrombin (TAT).

Heparin supports the TAT “approximation” reaction. When the UFH molecule exceeds 17 linear saccharide units, thrombin assembles on the molecule in approximation to (near) the activated AT. Approximation drives the TAT reaction at four times the rate of the AT-factor Xa reaction because Xa is inactivated only by antithrombin's protease binding site, independent of approximation.

UFH lots are unrefined and vary in MW, molecule length, and anticoagulant efficacy. Individual patient UFH metabolism rates diverge markedly because human plasma and cellular proteins bind heparin at varying rates and concentrations. Consequently, laboratory monitoring is essential to UFH therapy.

Physicians administer UFH intravenously to treat deep

vein thrombosis, pulmonary embolism, and in the initial treatment of acute myocardial infarction (AMI); to prevent reocclusion after stent placement; and to maintain vascular patency during cardiopulmonary bypass graft surgery with extracorporeal circulation. Therapy begins with a bolus of 60–80 units/kg to a maximum of 5000 units, followed by continuous infusion at 12–18 units/kg/hour (Table 1). Physicians discontinue UFH at 5 days to avoid heparin-induced thrombocytopenia with thrombosis (HIT), a severe, often fatal complication in which platelets are activated by an IgG antibody that binds the heparin-platelet factor 4 complex and activates platelets.

**Table 1.** Unfractionated heparin therapeutic sequence

Step	Action
PTT and platelet count prior to UFH therapy	Collect lavender-closure (EDTA) and blue-closure (citrate) blood, specimen perform “baseline” PTT and platelet count
At start of UFH therapy	Bolus of 60–80 units/kg to a maximum of 5000 units
Continue with UFH therapy	Continue with 12–18 units/kg/hour for duration of therapy
Second PTT and platelet count	Collect blue- and lavender-closure specimen 4–6 hours after completion of bolus, not more than 24 hours from start of therapy, perform PTT and platelet count
Adjust dosage	Adjust dosage (drip rate) to achieve laboratory-published PTT target therapeutic range, confirm with a second PTT within 6 hours of adjustment
Subsequent PTTs and platelet counts	Repeat PTT and platelet count every 24 hours throughout duration of therapy and adjust dosage. If the platelet count drops by more than 40%, even if it remains within the reference interval, suspect HIT and discontinue immediately
Discontinue UFH at 5 days	UFH for more than 5 days increases the risk of HIT

### Monitoring Unfractionated Heparin Therapy

Clinicians monitor UFH therapy closely using the partial thromboplastin time test (PTT) and platelet

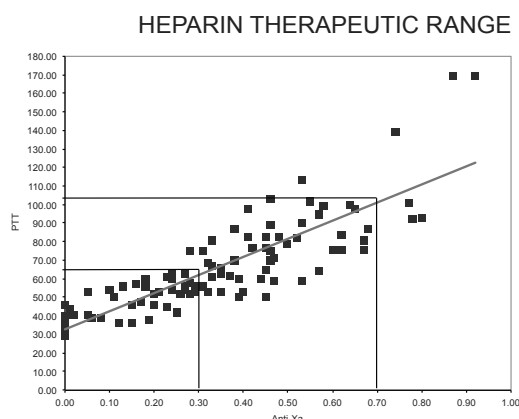
counts to avoid hemorrhage, rethrombosis, or heparin-induced thrombocytopenia with thrombosis (HIT). The phlebotomist collects a “baseline” blood specimen at the time the IV is started, carefully avoiding hemolysis. The specimen is assayed to ensure the non-heparin PTT is within the reference interval (RI); a prolonged baseline PTT may indicate the presence of a preexisting lupus anticoagulant, specific coagulation factor inhibitor, or factor deficiency, and confounds the therapeutic interpretation of the PTT result. In this instance, the laboratory scientist upgrades to the more reliable chromogenic anti-factor Xa heparin assay. A baseline platelet count is necessary to compare with later platelet counts, as a drop of more than 40%, even if the platelet count remains within the RI, signals the risk of HIT.

The phlebotomist collects a second specimen 4–6 hours after completion of the bolus administration, and not more than 24 hours from initiation of therapy and another PTT is performed. The result of the second specimen should fall within the therapeutic range, which is established in the laboratory (see the next section) and which is published with the result. The clinician adjusts the infusion rate to ensure the PTT result remains within the target range, repeating the PTT every 6 hours. Once the dosage is stable, the PTT is subsequently repeated every 24 hours until anticoagulation is discontinued. The laboratory scientist also monitors the platelet count daily. A 40% or greater reduction in platelet count, even if the count remains within the reference interval, is evidence for HIT. If HIT is suspected, UFH is immediately discontinued and replaced with pentasaccharide (fondaparinux) therapy or a direct thrombin inhibitor (DTI) such as argatroban.<sup>3</sup>

### Determining the Partial Thromboplastin Time Therapeutic Range

Hemostasis laboratory scientists establish and communicate a PTT therapeutic range for UFH therapy. The scientist collects 50 or more specimens from patients receiving UFH at all levels of anticoagulation and performs PTTs on all.<sup>4</sup> The specimens must be from patients who are not receiving Coumadin therapy; their PT results must be within the PT RI. Chromogenic anti-factor Xa heparin assays are performed on all specimens, and the paired PTT and anti-Xa results are displayed in a linear graph. The range

in seconds of PTT results that corresponds to 0.3–0.7 anti-factor Xa heparin units/mL is the therapeutic range. This is known as the *ex vivo* or “Brill-Edwards” method for establishing the heparin therapeutic range of the PTT and is required by proficiency testing and accreditation agencies (Figure 1).



**Figure 1.** Ex vivo “Brill-Edwards” curve to determine the UFH therapeutic range. PTTs and chromogenic anti-Xa assays are performed on at least 50 UFH specimens with normal PTs and on 20 normal subjects. Results are expressed as an XY graph. The PTT range in seconds that corresponds to 0.3–0.7 heparin anti-Xa units/mL is the target therapeutic range. A new Brill-Edwards curve is plotted with each change in PTT reagent lot.

The scientist reports the PTT result, the RI, and the current UFH therapeutic range. Because reagent sensitivity varies among producers and among individual producers’ reagent lots, the clinician must evaluate PTT results in relationship to the institution’s therapeutic range and RI, which may vary with each lot change. The PTT is typically used to measure the effects of UFH, however the chromogenic anti-factor Xa heparin assay may be used to assay UFH, low molecular weight (LMWH), and pentasaccharide (fondaparinux).<sup>5</sup>

### Limitations of the Partial Thromboplastin Time

Several interferences reduce PTT sensitivity, a circumstance called *heparin resistance*. (Table 2) Inflammation may be accompanied by hyperfibrinogenemia exceeding 400 mg/dL and von Willebrand factor or coagulation factor VIII activities over 150%. Both reduce the PTT’s response to heparin. Further, AT may become depleted in prolonged therapy or when there is an inherited or acquired underlying AT deficiency. The PTT result remains within the reference interval or is only slightly prolonged despite increasing

heparin dosages.

**Table 2.** Limitations of the PTT

“Heparin resistance;” PTT is insensitive to UFH therapy
• Hyperfibrinogenemia: fibrinogen exceeds 400 mg/dL in acute inflammation
• Coagulation factor VIII or von Willebrand factor over 150%
• AT becomes depleted during UFH therapy, congenital or acquired AT deficiency
Prolonged baseline PTT
• Hypofibrinogenemia: fibrinogen level below 100 mg/dL
• Congenital or acquired coagulopathy: single or multiple factor deficiency
• Specific factor inhibitor, most often factor VIII inhibitor
• Lupus anticoagulant: non-specific inhibitor
• Circulating fibrin degradation products or paraproteins

Hypofibrinogenemia, factor deficiencies, specific factor inhibitors, lupus anticoagulant, and the presence of fibrin degradation products or paraproteins prolong the PTT independent of heparin levels, rendering the assay overly sensitive and inaccurate for UFH monitoring.<sup>6</sup> In instances of resistance or prolonged baseline PTT, the laboratory scientist upgrades to the chromogenic anti-factor Xa heparin assay.

Platelets in anticoagulated blood specimens release platelet factor 4 (PF4), a heparin-neutralizing protein. In specimens from patients on UFH therapy, the PTT begins to shorten one hour after collection because of *in vitro* PF4 release. The specimen must be centrifuged to produce platelet-poor plasma with a platelet count below 10,000/ $\mu$ L, and the plasma must be removed from the cells. The PF4 interferes with both the PTT and the chromogenic anti-Xa heparin assay.<sup>7</sup>

### The Activated Clotting Time

The activated clotting time (ACT), a point-of-care assay, is used to monitor high-dose heparin therapy in the cardiology surgical suite.<sup>8</sup> ACT assay and instrument distributors provide evacuated specimen collection tubes that contain kaolin, a particulate clot activator. The fresh whole blood specimen is placed in the reaction well of, for instance, the Hemochron<sup>®</sup> Signature Elite (International Technidyne, Inc, Piscataway, NJ), where it is rotated and continuously monitored. When a clot forms, a magnet positioned within the sample is pulled away from a sensing device, stopping the timer. The RI of the ACT is typically 90–

175 seconds. The ACT is particularly useful for monitoring the high blood levels (1–2 units/mL) of UFH employed during coronary artery bypass surgery, which prolong the result to 200–400 seconds. Laboratory scientists seldom perform the ACT within the laboratory, but are called upon to assist with validation and troubleshooting of the operating room-based instrument.

### The Prothrombinase-induced Clotting Test

The prothrombinase-induced clotting test (PiCT<sup>®</sup>, Centerchem, Inc, Norwalk, CT; Pentapharm, Basel, Switzerland) employs a reagent composed of activated factor X (Xa), phospholipid, and Russell viper venom-V, a venom component that activates coagulation factor V.<sup>9</sup> The PiCT assay may be used to monitor UFH, LMWH, pentasaccharide, the oral direct anti-Xa anticoagulants rivaroxaban and apixaban, and the DTIs.

In the case of Heparin, LMWH and pentasaccharide, the scientist adds patient plasma to the reagent and incubates 3 minutes, during which time UFH-AT, LMWH-AT, and pentasaccharide-AT complexes are formed. In the case of direct anti-Xa drugs, the incubation time is omitted to avoid an AT effect that leads to an initial lowering of the clotting time at low concentrations of direct anti-Xa drug. This step results in an inhibition of a proportion of the FXa present in the reagent. The scientist next adds CaCl<sub>2</sub> and starts a timer. The residual Xa complexes with phospholipid and with the Va generated from the plasma by the RVV-V. The interval to clot formation is recorded and compared to a standard curve. The PiCT assay is under ongoing clinical studies to obtain FDA clearance as an aid in the monitoring of UFH therapy. Future studies will follow regarding all of the other drugs listed above. This test may prove to be the most versatile of the clot-based anticoagulant assays.

### Reversal of Unfractionated Heparin Using Protamine Sulfate

Protamine sulfate, a positively charged protein extracted from salmon sperm, neutralizes UFH at a ratio of 100 units of heparin per mg of protamine sulfate.<sup>10</sup> The physician administers protamine sulfate by slow intravenous push. The effect is detected by the instant shortening of the PTT or ACT. Protamine sulfate also neutralizes LMWH, although the neutralization is incompletely reflected in the results of the anti-factor

Xa heparin assay. Paradoxically, an overdose of protamine sulfate may cause hemorrhage.

### Low Molecular Weight Heparin

#### LMWH: Depolymerized Unfractionated Heparin

Uncertainty about UFH dose response led to the development of LMWH, which was cleared for prophylaxis in 1993. In the US, enoxaparin (Lovenox<sup>®</sup>, Aventis Corp) commands the major market share of LMWH preparations. LMWH is prepared from UFH using chemical or enzymatic fractionation. Fractionation yields a median molecular weight of 4500–5000 Daltons, about one third the mass of UFH. LMWH possesses the same active pentasaccharide sequence as UFH; however, the overall shorter polysaccharide chains provide somewhat less space for thrombin approximation, so the AT to thrombin neutralization response is reduced. The AT to factor Xa neutralization response is unchanged, however, as the Xa reaction does not rely on approximation, so LMWH provides nearly the same anticoagulant efficacy as UFH, predominantly through Xa inhibition.

Patients self-administer LMWH by subcutaneous injection once or twice a day using premeasured syringes at selected dosages, for instance, 30 mg every 12 hours or 40 mg once daily. Prophylactic applications provide coverage during or after neurosurgery and orthopedic surgery and after trauma, typically for 14 days from the time of the event. Hematologists also use LMWH to treat DVT, PE, and unstable angina. When Coumadin patients require surgery, Coumadin is discontinued for up to a week before the procedure and replaced with LMWH, which has a shorter half-life, produces less risk of bleeding, and may be partially reversed with protamine sulfate.

LMWH's advantages are rapid bioavailability after subcutaneous injection, making IV administration unnecessary; half-life of 3–5 hours compared with 1–2 hours for UFH; and a fixed dose response that reduces the need for laboratory monitoring. The risk of HIT is reduced by 90% in people who have never received heparin before, however LMWH reacts with previously formed HIT antibodies. The risk of LMWH-induced bleeding is equivalent to UFH, about 10%.

### Laboratory Assay of Low-Molecular-Weight Heparin

LMWH is cleared by the kidneys alone, so it



accumulates in renal insufficiency. A laboratory assay is necessary when the glomerular filtration rate (GFR) is less than 30 mL/min or the serum creatinine exceeds 4 mg/dL (Table 3). Creatinine assays are run periodically to document kidney function and avoid the risk of LMWH accumulation.

To monitor LMWH, the phlebotomist collects a specimen 4 hours after subcutaneous injection and the platelet-poor plasma is tested using the anti-factor Xa heparin assay, which employs a fixed concentration of factor Xa and a chromogenic substrate specific to the enzymatic properties of Xa. LMWH forms a complex with AT that may be supplied by the reagent or may be provided by the patient plasma. The LMWH-AT complex inactivates reagent factor Xa. A measured excess of factor Xa digests the substrate, yielding a colored product whose intensity is inversely proportional to the initial heparin concentration.

**Table 3.** Reasons for performing a laboratory assay of low molecular weight heparin

It is not necessary to monitor LMWH therapy routinely, however it must be assayed when there is a fluid imbalance or when the coagulation system is unstable:

- Renal disease: glomerular filtration rate less than 30 mL/min or serum creatinine exceeds 4 mg/dL
- Abnormal blood and cellular fluid distribution in morbid obesity or in the excessively slender
- People over 70 whose population was not included in clinical trials
- Children
- Pregnancy
- Cancer
- Diabetes
- Chronic inflammation
- Liver disease

To prepare a standard curve, the laboratory scientist obtains a characteristic lot of LMWH from the pharmacy and prepares dilutions that correspond with the intended therapeutic range. If the chromogenic anti-factor Xa heparin assay is to be used to monitor UFH and LMWH, a single hybrid standard curve may be prepared.<sup>11</sup> A separate curve may be necessary to monitor pentasaccharide (fondaparinux). The therapeutic range for twice-daily LMWH regimens is 0.5–1 units/mL and for once-daily regimens is 1–2 units/mL.

The anti-factor Xa heparin assay and the PiCT are the

only assays available to monitor LMWH and pentasaccharide therapy. They may also be used in place of the PTT to assay UFH with little or no modification and may substitute for the PTT when clinical or laboratory conditions render the PTT unreliable. The chromogenic anti-factor Xa heparin assay is also the reference method for establishing the PTT therapeutic range in the Brill-Edwards curve procedure, discussed previously.

### Pentasaccharide (Fondaparinux) Therapy

Fondaparinux sodium (Arixtra<sup>®</sup>; GlaxoSmithKline, Research Triangle Park, NC) is a synthetic formulation of the active pentasaccharide sequence in UFH and LMWH. Fondaparinux is equivalent in clinical efficacy and safety to UFH and LMWH, has a reproducible dose response and a half-life of 12–17 hours, requiring once-a-day subcutaneous injections of 2.5 mg each. Fondaparinux is approved for surgical prophylaxis and for the treatment of DVT and PE but is contraindicated for patients with a GFR less than 30 mL/min or with body weights less than 50 kg.<sup>12</sup>

Scientists employ the PiCT or the chromogenic anti-factor Xa heparin assay to monitor fondaparinux therapy when necessary. Blood is collected four hours after injection, and the target range is 0.14–0.19 mg/L. The laboratory scientist prepares a standard curve using fondaparinux, not UFH, LMWH, nor a hybrid standard, because concentrations are expressed in mg/L and not units/dL.

### Rivaroxaban and Apixaban, Oral Direct Xa Inhibitors

Oral rivaroxaban (Xarelto<sup>®</sup>; Bayer Healthcare AG, Leverkusen, Germany; Ortho-McNeil Pharmaceuticals, Inc, Raritan, NJ) and oral apixaban (Eliquis<sup>®</sup>, Bristol-Myers Squibb and Pfizer, New York, NY) function unmodified to inhibit Xa, bypassing the need for AT. Both have favorable efficacy and safety outcomes when compared to UFH, LMWH or Coumadin.<sup>13</sup> Both appear to provide attractive alternatives to Coumadin and LMWH injections because they are convenient and require little laboratory monitoring. Rivaroxaban was FDA-cleared July 1, 2011 for VTE prophylaxis in patients who are undergoing total knee replacement or total hip replacement surgery. Apixaban was cleared in December, 2012.<sup>14</sup> The standard oral rivaroxaban dosage is 10 mg/day and because of its predictable pharmacodynamics, laboratory monitoring is seldom

necessary. Rivaroxaban and apixaban prolong the PT and PTT, as reported in several clinical trials. PTT responses vary widely among reagents and reagent lots, thus no attempt has been made to monitor either anticoagulant with the PTT. The PT, however, provides a reproducible linear relationship with rivaroxaban.<sup>15</sup> Rivaroxaban (and by generalization, apixaban) may also be assayed using the chromogenic anti-Xa heparin assay by standardizing with rivaroxaban (or apixaban) in place of UFH, LMWH or fondaparinux, though it remains for laboratory scientists to correlate laboratory results with clinical outcomes. In September, 2012, Diag-nostica Stago, Inc announced the availability of rivaroxaban control and calibrator plasmas to be used with their chromogenic anti-Xa kit.

The pharmaceutical industry and the in vitro diagnostics industry have together made significant strides, beginning with crude UFH that is typically administered intravenously, LMWH and pentasaccharide in predictable doses administered subcutaneously, and now rivaroxaban and apixaban, both safe and effective oral preparations. We've made modest progress towards drugs that are both effective and safe, threatening to topple Coumadin and heparin from their lofty perches, however there are three wrinkles left to iron out. No laboratory assay has been FDA-cleared for monitoring rivaroxaban or apixaban. All current kits, controls, and calibrators are labeled for research use only. While safe and effective, the anticoagulant properties of rivaroxaban and apixaban have both rapid onset and rapid regression. Consequently, compliance must be 100%; one missed dosage places the patient in danger of rethrombosis. And, unlike UFH and LMWH, no effective reversal agent has been developed for fondaparinux, rivaroxaban, or apixaban, thus leaving the emergency department with only uncertain solutions for patients at risk for an intracranial hemorrhage. These issues are soon to be resolved, revolutionizing the field of anticoagulation.

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