REVIEW ARTICLE

Unfractionated heparin: optimizing laboratory monitoring and reducing unwanted interference in everyday hemostasis test practice

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KEY WORDS

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ABSTRACT

Unfractionated heparin (UFH) serves as a commonly used anticoagulant. It is widely utilized for a variety of reasons, including to 1) anticoagulate patients and help treat and/or prevent thrombosis, 2) maintain patency in artificial blood flow circuits, and 3) anticoagulate blood samples collected for laboratory testing (typically for biochemical assays or blood gas analysis). As such, the presence of UFH is nearly ubiquitous in a hospital setting. Therefore, in laboratory practice, UFH may be present in samples intended for monitoring patients on UFH therapy or intended for biochemical tests, or it may interfere with other (hemostasis) laboratory tests. The aim of this manuscript is to review the role of UFH from the perspective of optimizing laboratory testing to monitor UFH therapy and to avoid or overcome unwanted interference with other laboratory tests.

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Introduction Unfractionated heparin (UFH) is commonly used as an anticoagulant. UFH represents a total fraction of heparin derived from sources such as porcine mucosa,[1] and its molecular weight ranges from 5000 to 30 000 Da, with an average of 15 000 Da.[2] Contrary to that, low-molecular-weight heparin (LMWH), as its name implies, is a subfraction of UFH, with average molecular weight around 4500 Da.[2] LMWH is also commonly used as an anticoagulant. UFH and LMWH may be differentially administered to patients, depending on clinical or therapeutic considerations.[2] In addition, UFH may be used for other reasons.

UFH is widely employed in 1) anticoagulation of patients to help treat and/or prevent thrombosis, 2) maintenance of patency in artificial blood flow circuits (eg, extracorporeal membrane oxygenation [ECMO]), and 3) anticoagulation of blood samples collected for laboratory testing (most typically for selected biochemical assays or blood gas analysis) (TABLE 1). Accordingly, UFH is fairly common in the hospital setting. Therefore, a chance that a patient's sample contains heparin is high in laboratory practice. In fact, UFH may be present in samples used for monitoring patients on UFH therapy,[2,3] but it may also undesirably interfere with many laboratory tests.[4]

The aim of this manuscript is to review UFH from the perspective of optimizing laboratory testing. We will consider both monitoring of UFH therapy and methods to avoid unwanted interference in laboratory tests, or, if avoidance is not possible, methods to overcome such interference.

Unfractionated heparin for patient treatment and monitoring UFH is a relatively common and inexpensive drug widely used to treat patients with recent thrombosis and/or to prevent thrombosis recurrence.[2,5,6] UFH is typically given by parenteral

TABLE 1 Sources of heparin in laboratory samples

Source of heparin	Intended use	Effect on hemostasis assay	Solution/action
UFH for prophylaxis	Prevention of VTE in medical and surgical patients	• Prolongation of APTT and TT • Interference with any assay depending on anti-Xa or anti-IIa activity	• Clinicians should advise a laboratory of patient's treatment • The laboratory shoud recognize UFH as an assay-interfering factor
UFH for treatment	Treatment of VTE/prevention of recurrence	• Prolongation of APTT and TT • Prolongation of dRVVT and PT if UFH level exceeds heparin neutralizing ability of a reagent • Interference with any assay depending on anti-Xa or anti-IIa activity	• Clinicians should advise a laboratory of patient's treatments • Monitor UFH by APTT or anti-Xa assay
LMWH for prophylaxis	Prevention of VTE in medical and surgical patients	• Mild prolongation of APTT • Interference with any assay depending on anti-Xa activity	• Clinicians should advise a laboratory of patient's treatment • The laboratory should recognize LMWH as an assay-interfering factor
Other treatments (ACS, PCI, STEMI, non-STEMI, unstable angina)	Prevention of coronary artery intraluminal thrombus extension and recurrence	• Prolongation of APTT and TT • Interference with any assay depending on anti-Xa or anti-IIa activity	• Clinicians should advise a laboratory of patient's treatment • Monitoring with APTT or anti-Xa may be indicated
Heparin for maintaining catheter patency, flushing catheters, ECMO, dialysis, etc.	Prevention of thrombosis or blockage in a catheter or artificial blood flow circuits	• Prolongation of APTT and TT • Prolongation of dRVVT and PT if UFH level exceeds heparin neutralizing ability of a reagent • Interference with any assay depending on anti-Xa or anti-IIa activity	• Clinicians should advise a laboratory of patient's treatment • The laboratory should recognize heparin as an assay-interfering factor
Sodium heparin anticoagulated blood collection tubes	Measurement of certain biochemical parameters (eg, lithium)	• General prolongation of all clot-based assays • Interference with any assay depending on anti-Xa or anti-IIa activity	• Blood collectors should not top up underfilled citrate blood collection tubes with blood from other collection tubes • The laboratory should run tests for sodium level if contamination with sodium heparin is suspected
Lithium heparin anticoagulated blood collection tubes	Measurement of certain biochemical parameters (eg, electrolytes)	• General prolongation of all clot-based assays • Interference with any assay depending on anti-Xa or anti-IIa activity	• Blood collectors should not top up underfilled citrate blood collection tubes with blood from other collection tubes • The laboratory should run tests for lithium level if contamination with lithium heparin is suspected

Abbreviations: ACS, acute coronary syndrome; anti-IIa, antithrombin assay; anti-Xa, anti–activated factor X assay; APTT, activated partial thromboplastin time; dRVVT, diluted Russell viper venom time; ECMO, extracorporeal membrane oxygenation; LA, lupus anticoagulant; LMWH, low-molecular-weight heparin; PCI, percutaneous coronary intervention; PT, prothrombin time; STEMI, ST-segment elevation myocardial infarction; TT, thrombin time; UFH, unfractionated heparin; VTE, venous thromboembolism

route (ie, injected intravenously [IV] as sodium heparin] or subcutaneously [SC] as calcium heparin], depending on the clinical indication).[7] For example, UFH may be given for prophylaxis to medical or surgical patients at a risk of venous thrombosis (typically SC at smaller fixed doses without a need for laboratory monitoring) or administered due to a recent venous thromboembolic event and to prevent its recurrence (typically IV at higher doses and with laboratory monitoring). For the latter, UFH is usually used to initiate anticoagulation, with the aim of transition to another, typically oral, anticoagulant for long-term management. This transition may be to a vitamin K antagonist (VKA), for example warfarin or other coumarins, or to a direct oral anticoagulant (DOAC), such as dabigatran, rivaroxaban, apixaban, or edoxaban. Furthermore, UFH is used in management of acute coronary syndromes to prevent coronary artery intraluminal thrombus extension and recurrence.[8]

When indicated, laboratory monitoring of UFH therapy is typically accomplished using either an activated partial thromboplastin time (APTT) or an anti-factor Xa (anti-Xa) assay.[3,9-12] In either case, these assays are based on established therapeutic intervals (therapeutic reference range [TRR]) to maintain patients within a safe therapeutic UFH range, since too low a level of UFH poses a risk of VTE recurrence, and too high of bleeding.[3,10] For the chromogenic anti-Xa assay, the most common TRR used is 0.3–0.7 U/ml. For the APTT, the most common TRR is based on local equivalence (ie, using the local type of APTT reagent) to the range of 0.3–0.7 U/ml in the anti-Xa assay. The presence of UFH may also undesirably interfere with other clotting assays. Similarly, factors other than UFH may affect APTT and anti-Xa assays.[3,9-12]

Unfractionated heparin for catheter and circuit patency UFH is also widely used for maintaining

TABLE 2 Advantages and limitations of monitoring with anti-factor Xa assay vs activated partial thromboplastin time

Assay	Advantages/benefits	Limitations/disadvantages
Anti-Xa assay	• Does not require establishing a TRR, which is assumed to be 0.3–0.7 U/ml • More direct measurement of heparin anti-Xa activity than APTT • Can also be used to measure LMWH and other anti-Xa agents, including DOACs (eg, rivaroxaban, apixaban, edoxaban) • Not affected by the presence of LA or factor deficiency (eg, FXII) • Not affected by the presence of acute phase proteins (eg, fibrinogen, FVIII)	• More expensive than APTT • Small laboratories may not be able to utilize test reagents before they expire (high wastage) • Not specific for UFH, LMWH, or DOAC (ie, the presence of any anti-Xa agent influences the assay) • Requires assay-specific calibrators for UFH, LMWH, rivaroxaban, apixaban, or edoxaban according to the measured drug • Does not measure anti-IIa activity • Different anti-Xa methods may or may not include exogenous dextran sulphate or antithrombin, which may result in different sensitivity of the assay to heparin
APTT	• Cheaper to perform than anti-Xa assay • Once established for a particular reagent lot, can utilize the same TRR across a network of laboratories using the same reagent lot	• Requires establishing of an APTT TRR that is equivalent to an anti-Xa TRR of 0.3–0.7 U/ml • Requires checking and potential re-establishing of the APTT TRR with each change of the reagent lot • Less direct measure of heparin activity than anti-Xa assay • Affected by many other drugs, factor deficiencies (eg, FXII) or inhibitors (eg, LA), compromising accuracy of heparin monitoring

Abbreviations: DOAC, direct oral anticoagulant; F, factor; LA, lupus anticoagulant; TRR, therapeutic reference range; others, see TABLE 1

catheter patency, and preventing clot formation and/or blockage within a variety of arterial and venous catheters.[13-15] Here, UFH may be used to fill a catheter or flush it between uses. UFH is also commonly used as an anticoagulant during renal dialysis[16] or ECMO treatment,[17,18] that is, a form of life support for people with life-threatening illnesses or injuries that affect functioning of their heart or lungs. In some situations, patients may be on ECMO for months. For such applications, UFH level is not normally monitored, but its presence in blood should be considered for assessment of various hemostasis parameters, since it may generate unwanted interferences in laboratory assays.

Heparin and COVID-19 COVID-19 is a widely acknowledged prothrombotic condition, with hospitalized patients commonly anticoagulated, usually with heparin.[19] Of particular relevance to the readership of this paper are recent recommendations on the management of COVID-19, including heparin therapy, recently published by the Polish Association of Epidemiologists and Infectiologists.[20]

Unfractionated heparin and blood collection UFH is commonly used for collecting blood for blood gas analysis. Lithium heparin concentration in these samples may be as high as 40 IU/ml. Thus, small amounts of blood contaminated with heparin appear when needles are subsequently used for collection of blood for other tests, especially hemostasis assays. These amounts may act as interferents in these other assays. Sodium heparin blood collection tubes may alternatively be used for testing lithium levels, some immunologic assays, cytogenetic assays, and flow cytometry tests. Lithium heparin may be used for blood collection for some clinical chemical and trace element testing, including sodium levels. The heparin concentration in these blood collection tubes can be as high as 10 IU/ml. If heparinized blood is included in a sample intended for testing in a hemostasis or coagulation laboratory, it may interfere with an assay and generate unreliable test results.[21] Heparinized samples are also typically unsuitable for hematologic testing, as heparin interferes with most staining reagents, causing cells to clump and invalidating blood counts.

Laboratory monitoring of unfractionated heparin therapy As mentioned above, UFH therapy can be monitored using either the anti-Xa or APTT assay. There are advantages and limitations to either approach.[3,9-13] The anti-Xa method is considered superior and more advantageous than the APTT for several reasons (TABLE 2). First, it is a more direct method of assessing UFH activity, and does not require establishing a TRR. The assays are available from most manufacturers of hemostasis reagents, and the established TRR is generally taken to be 0.3–0.7 U/ml. This TRR does not change with different lots of the test reagents. The assays can be fully automated and run in continuously operating (24 hours/day, 7 days/week) laboratories. Each assay includes a calibration curve, derived from a calibrated reference sample supplied with the assay kit, from which a patient's UFH level can be estimated.

The anti-Xa method is also less susceptible to assay interference, as, in contrast to APTT, it is not influenced by the presence of lupus anticoagulant (LA), factor (F) deficiency (especially

FXII), or acute phase proteins, such as fibrinogen and FVIII. Both LA and mild FXII deficiency are fairly common causes of APTT prolongation. On the other hand, the presence of acute phase proteins is also a potential cause of APTT shortening; these proteins are commonly released during infection or inflammation. In such cases, the effect on the APTT may be so called "heparin resistance,"[22] since more heparin is required to raise the APTT to a within-therapy range. This may be particularly relevant in the intensive care unit setting. In general, the anti-Xa assay is sufficient for heparin monitoring without a need for APTT testing, unless there are concerns regarding test results, and when APTT serves as a second, confirmatory measure of heparin effect. If the anti-Xa assay is not available, the APTT method can be used.

Theoretical disadvantages of the anti-Xa method include its higher costs of individual procedures than of standard APTT assays. However, the cost is even higher if UFH monitoring is infrequently performed at a given site. In such cases, the assay kit may expire before the reagent is fully utilized; thereby, the cost per test is considerably higher than the theoretical cost based on feasible tests / kits. Also, anti-Xa testing only measures the anti-Xa activity of heparin, and does not measure anti-IIa activity, with UFH having an anti-Xa to anti-IIa ratio of approximately 1:1. In contrast, the APTT assay provides a measure of anti-IIa activity. Importantly, different anti-Xa methods may be available, and they may yield slightly different results. In particular, some methods involve dextran sulphate or require an addition of exogenous antithrombin, whereas others do not. In the presence of high concentrations of dextran sulphate, FXa inhibition is much stronger than that predicted from added UFH amounts,[23,24] which is presumably related to greater availability of UFH for interaction with antithrombin in the assay. As for antithrombin, its inclusion in an antithrombin assay may artificially increase the perceived anti-Xa effect, and may not accurately reflect actual in vivo anticoagulation, especially if a patient has reduced antithrombin levels. Finally, the accepted therapeutic range of 0.3–0.7 U/ml derives from small historic studies, and has never been validated in any large clinical trial.[3,10] Nevertheless, the anti-Xa method is generally preferred in larger facilities that perform frequent UFH monitoring.

It can also be noted that the anti-Xa assay is not specific to UFH, and can be potentially used for monitoring or measuring the level of any anti-Xa therapeutic agent, including LMWH, and direct anti-Xa agents, such as rivaroxaban, apixaban, and edoxaban.[25,26] For such purposes, the anti-Xa assay is calibrated with a specific drug of interest, and optimized to be linearly sensitive to expected TRRs or "within therapy" ranges. Nevertheless, each anti-Xa agent can be seen as an assay-interfering factor when a different anti-Xa agent is being measured for the purpose of measuring drug levels. In other words, should a patient be transitioned from heparin to a direct anti-Xa agent, the anti-Xa level will reflect a combination of heparin and direct anti-Xa agent activity, and thus the test results of the anti-Xa assay (eg, calibrated to heparin) will be inaccurate. Naturally, the same applies to the APTT, that is, its total prolongation reflects a composite of anticoagulant effects.

In contrast with the anti-Xa method, the APTT method requires establishing a TRR for every APTT reagent, since the sensitivity of an APTT reagent to heparin differs according to both the local reagent and instrument used.[9,11,27] This TRR also requires check-up and potential revision with every change to the APTT reagent lot. Such changes cause substantial problems for clinical, nursing, or pharmacy staff monitoring the patients on UFH, since corrections to established dosing algorithms and ongoing staff retraining may be required. As the range of 0.3–0.7 U/ml associated with the anti-Xa testing, the TRRs of APTT have also been established based on small historic studies, and have never been validated in any large clinical trial.[10] As also noted previously, the APTT may be affected by a wide range of factors unrelated to heparin, increasing in the presence of LA and factor deficiencies (eg, FXII), and decreasing in the presence of acute phase proteins, such as fibrinogen and FVIII. As all these affect baseline APTT values, addition of heparin may lead to excessive APTT of expected TRRs in the first scenario, and the effect of heparin resistance in the second scenario.

An advantage of the APTT assay is its generally lower cost than of the anti-Xa one. It also captures anti-IIa activity, and thus yields a composite of anticoagulation for UFH monitoring. Thus, thanks to its more general use, the APTT method may be preferred in smaller or satellite laboratories, and the APTT may also be used in some laboratories as a screen of hemostasis (eg, to detect factor deficiencies or identify LA), or to monitor disseminated intravascular coagulation.[9] On the other hand, this broader sensitivity may confound the results of the APTT assays for UFH monitoring. For example, patients with LA and with FXII deficiency may need to be monitored when on UFH therapy, and for them the APTT is generally not suitable, since their baseline APTT is elevated, and the use of standard therapeutic intervals will generally lead to suboptimal anticoagulation and increased risk of thrombosis.

In summary, the anti-Xa assays can be considered advantageous over the APTT testing in all clinical settings, ensuring that the test results can be provided to a requesting clinician in a timely manner.

Heparin resistance Heparin resistance reflects a state in which more heparin than expected needs to be administered to a patient to achieve a test result within an expected therapeutic range.[22] For APTT-based monitoring, the most

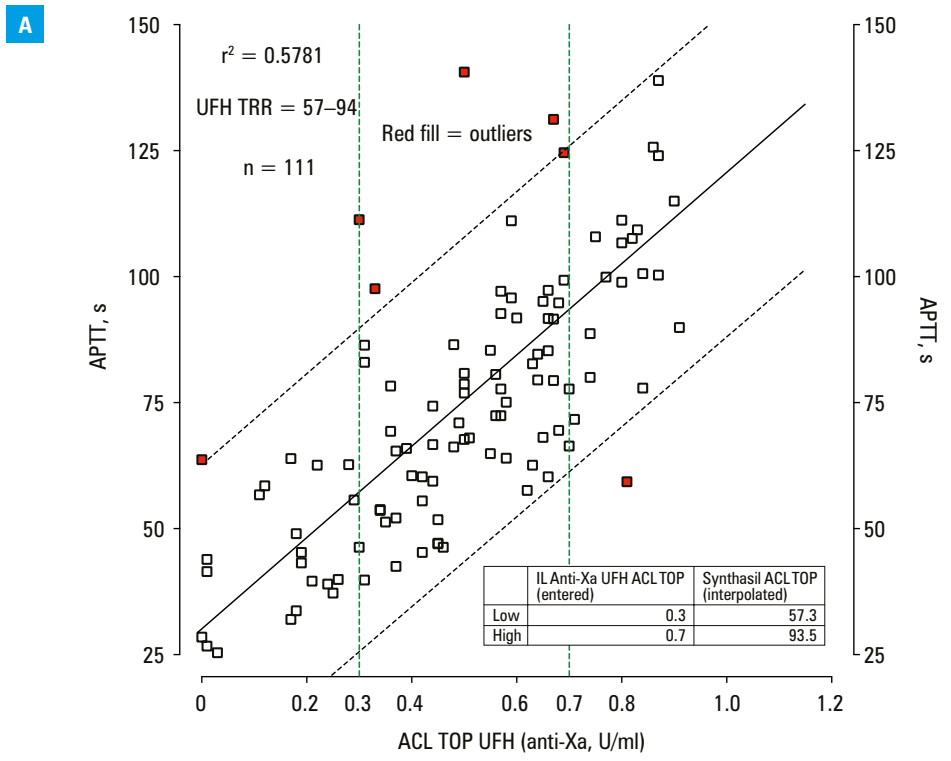
common causes of heparin resistance is the presence of acute phase proteins (such as fibrinogen and FVIII) due to an infection or inflammation. In such cases, their presence may decrease the baseline APTT, and more heparin is needed to bring the APTT into the TRR. An alternative form of heparin resistance that may affect the anti-Xa assay is the presence of antithrombin deficiency, especially if the anti-Xa kit does not include exogenous antithrombin, and as heparin is an indirect anticoagulant requiring the presence of antithrombin to generate anti-Xa activity.

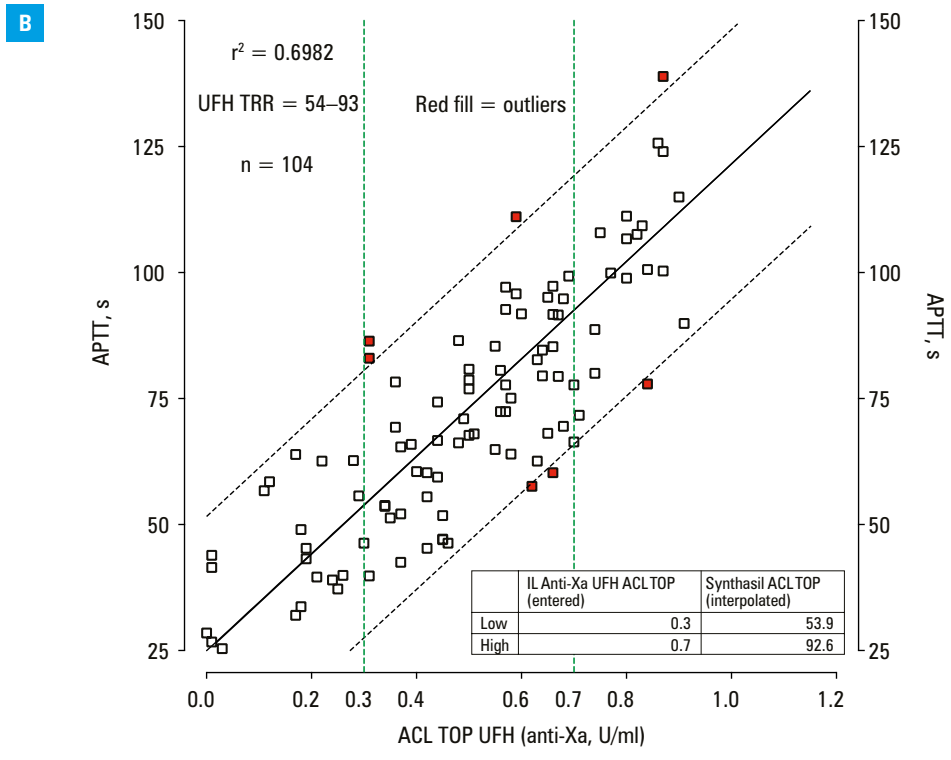
To evaluate these possibilities in individual patients, further testing may be required (ie, fibrinogen and FVIII testing in the case of APTT-based heparin resistance, and antithrombin testing in the case of anti-Xa–based heparin resistance). In the case of the APTT-based heparin resistance, preferential patient assessment with the anti-Xa method is recommended. In the case of anti-Xa–based heparin resistance, consideration should be made to increase antithrombin levels if antithrombin deficiency is found.[22]

Optimizing laboratory unfractionated heparin monitoring with the anti-Xa method Although the anti-Xa assay is a more direct measure of heparin activity, and is less affected by interferences that may affect the APTT, it is not specific to heparin. The assay is made more specific for any agent by using specific calibrators, but any drug that expresses anti-Xa activity interferes with the assay results and thus potentially compromises its utility for specific drugs. Thus, UFH, LMWH, fondaparinux, and other anti-Xa agents, including some DOACs (rivaroxaban, apixaban, edoxaban), influence the test results, and the accuracy for a given drug effect is only guaranteed when a patient is only on that drug, and does not take any other anti-Xa agents. Moreover, blood for this assay is collected into sodium citrate anticoagulant tubes, and any contamination of the blood with heparin-anticoagulated samples must be avoided, so as to accurately assess the patient's heparin level. This includes situations in which inexperienced clinicians, seeing an underfilled citrate anticoagulant tube that may be rejected by a laboratory, top up that blood tube with blood from another tube to make it acceptably full for the laboratory.

Optimizing laboratory unfractionated heparin monitoring with the activated partial thromboplastin time method Each APTT reagent used to monitor heparin therapy requires establishing the APTT TRR.[9-11] APTT reagents differ in their sensitivity to heparin, and to be used for heparin monitoring they have to have good linear sensitivity to UFH over the expected TRR reflected by 0.3–0.7 U/ml in the anti-Xa assay (or heparin level of 0.2–0.4 U/ml in protamine sulfate titration may be used in some laboratories). The use of different instruments also influences the TRR.[27] To establish the TRR, a laboratory needs an access to patients undergoing UHF therapy. As many other factors affect the APTT, the assessment must avoid using samples from patients on other anticoagulant therapies (including VKAs or DOACs), patients with liver disease, or those with factor deficiencies (especially FXII), and also those with LA. For example, it is common to select samples from patients with normal prothrombin time (PT) or international normalized ratio (INR) test results, and preferably also with normal baseline (preheparin) APTT test results. The number of patient samples required to establish a TRR is debatable, but at least 20 are required.[28] The more patient samples are used, the more accurate the established TRR for UFH monitoring. These patient samples are tested with both the APTT and anti-Xa assays, and the results are plotted. Outlier data points are removed, and the data are replotted.[9,29] An example from one of our laboratory networks is shown in FIGURE 1, with workflow explained in the figure legend. In this network-based analysis, several rounds of outlier removal were undertaken to improve the relationship before settling on a final TRR.

Minimizing heparin interference in hemostasis testing While during laboratory monitoring of heparin therapy the presence of heparin in a blood sample is expected, the drug interferes with a range of hemostasis assays (TABLE 3). The best option for assessing hemostasis in such patients is to avoid heparin contamination, including possible contamination from heparin therapy, heparin flushes, or heparinized blood. Some hemostasis reagents are manufactured to be insensitive to therapeutic heparin levels. For example, PT reagents often contain heparin neutralizers to make them insensitive to therapeutic levels of heparin and thus more appropriate to assess specific VKA effects (ie, when used as the INR for monitoring VKA therapy). Diluted Russell viper venom time reagents used to assess a potential presence of LA are also generally made insensitive to therapeutic levels of heparin. This is highly desirable, as LA is often assessed in patients after a thrombotic event, and possibly on heparin therapy at the time of LA testing. Unfortunately, since APTT reagents may be also used to monitor UFH therapy, UFH interference becomes problematic when assessing LA. Although it may be ideal to have manufacturers produce different versions of the APTT reagents (ie, sensitive to UFH for UFH level monitoring and insensitive to UFH for LA testing), such reagents are currently unavailable. Instead, heparin can be neutralized with $CaCl_2$ containing a heparin neutralizer.[30] A laboratory can have 2 different $CaCl_2$ solutions to use with their APTT reagents—one without a heparin neutralizer, which can be used for UFH monitoring, and one with the heparin neutralizer, which can be used for LA testing. Finally, it is crucial that clinicians inform laboratories about the anticoagulation status of their patients when they order hemostasis assays. Indeed, we


A
r² = 0.5781
UFH TRR = 57–94
n = 111
Red fill = outliers
APTT, s
ACL TOP UFH (anti-Xa, U/ml)
IL Anti-Xa UFH ACL TOP (entered)
Synthasil ACL TOP (interpolated)
Low 0.3 57.3
High 0.7 93.5


B
r² = 0.6982
UFH TRR = 54–93
n = 104
Red fill = outliers
APTT, s
ACL TOP UFH (anti-Xa, U/ml)
IL Anti-Xa UFH ACL TOP (entered)
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Low 0.3 53.9
High 0.7 92.6

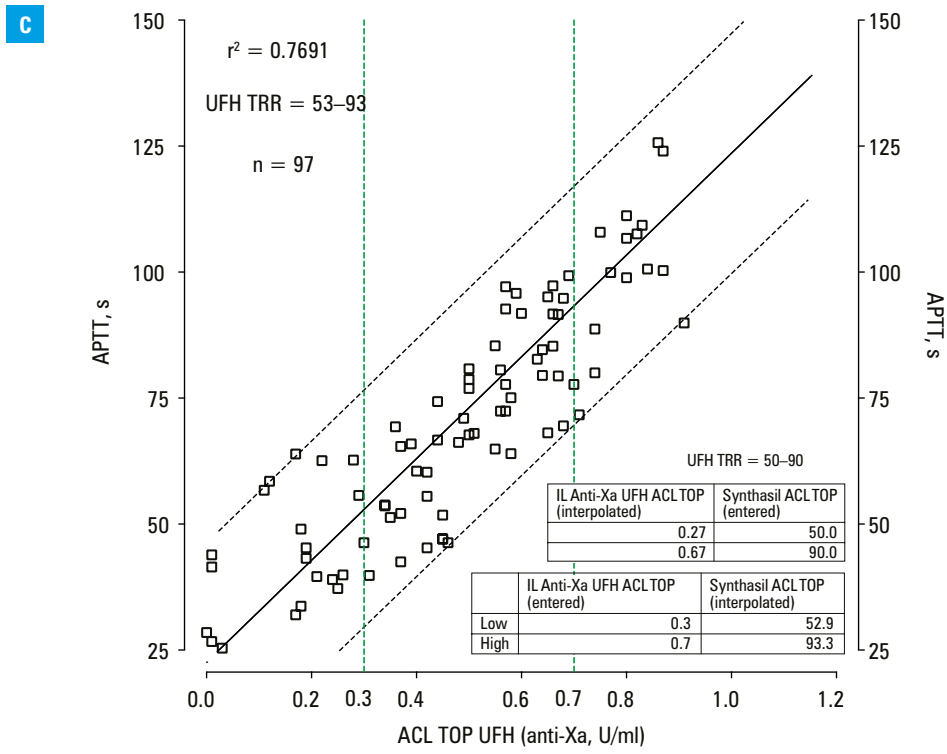

FIGURE 1 An example of establishing activated partial thromboplastin time (APTT) therapeutic reference range (TRR) for unfractionated heparin (UFH). These data reflect the use of data points from several centers within New South Wales Health Pathology to enable optimized assessment. In the first round, all potentially suitable patient sample data points identified for patients on UFH are assessed for both APTT and anti-factor Xa in a chromogenic assay. Only data from the patients with normal prothrombin time and international normalized ratio (<1.2) are included to avoid capturing the patients on vitamin K antagonist therapy. Also, samples should preferably be collected from patients with originally normal baseline APTT values. Data from several partner sites permit inclusion of more data points, especially considering difficulties with obtaining sufficient data points from a single site. The data points are preferably tested fresh (ie, without freezing/thawing). However, the data points can be supplemented with frozen/thawed plasma samples (eg, archived material), providing that the samples undergo double centrifugation to remove platelets, which if included release heparin-neutralizing components (eg, platelet factor 4) and phospholipids that may promote coagulation activation. Despite this, some outlier data are evident in the first round (**A**) (red symbols). These may indicate samples with unrecognized coincident factor deficiency (eg, factor XII) or lupus anticoagulant resulting in unexpectedly high APTTs, or patients actually on low-molecular-weight heparin instead of UFH, resulting in unexpectedly low APTT despite high anti-Xa levels. These outliers should be removed and the data should be re-evaluated. For them, a second data cleanup (**B**) was performed, leaving the final data set (**C**). The effect of this sequential data analysis is summarized in **TABLE 4**. With each step, the relationship improves (increasing r or r^2 values). Interestingly, however, the calculated TRR does not change much.

mandate such disclosure in our Westmead laboratory for electronic orders.

Education of blood collection staff is also mandatory. Professional phlebotomists tend to be well educated and experienced, and generally know that each blood collection tube has a particular purpose, and do not mix blood from different collection tubes. Inexperienced blood collectors, especially clinicians under training, may be less knowledgeable. In particular, if in the past they supplied a laboratory with an underfilled sodium citrate blood collection tube and the laboratory consequently cancelled the tests, such inexperienced collectors may be tempted to ensure that they fill the underfilled sodium citrate blood collection tube with blood from another tube. Collectors should be trained to simply never do this. Topping up underfilled sodium citrate blood collection tubes with blood from another tube inevitably affects clot-based assays. Even topping up with blood from another sodium citrate blood collection tube may lead to an excess of citrate anticoagulant. Consequently clot test times may be falsely prolonged, and discrete analytes, such as factors may be diluted, yielding false low levels. Topping up an underfilled sodium citrate blood collection tube with blood from a heparin anticoagulated blood collection tube results in high heparin levels in the topped-up tube, prolongation of most clot-based assays, as well as interference with any assay that depends on the anti-Xa or anti-IIa (thrombin) activity. It is nearly impossible to neutralize such high levels of heparin to enable hemostasis assays to be performed,

TABLE 3 Common hemostasis assays affected by heparin

Assay	Solution
APTT, if used for any other purposes than monitoring UFH levels (eg, for LA detection/exclusion)	• Utilize heparin neutralizer in a $CaCl_2$ solution • Manufacturers can make specific APTT reagents for LA detection/exclusion and for UFH monitoring
dRVVT (if UFH is above the therapeutic range)	• Awareness of this potential issue • UFH level can be checked with an anti-Xa assay
PT (if UFH is above the therapeutic range)	• Awareness of this potential issue • UFH level can be checked with an anti-Xa assay
Antithrombin assays	• Awareness of this potential issue • UFH level can be checked with an anti-Xa assay
TT, unless used as a marker of potential UFH use	• Awareness of this potential issue • UFH level can be checked with an anti-Xa assay

Abbreviations: see TABLES 1 and 2

TABLE 4 Generating an activated partial thromboplastin time therapeutic reference range for unfractionated heparin[a]

Setting	Number of data points	r and r^2 values	Calculated APTT TRR, s
Initial data capture (FIGURE 1A)	111	0.760; 0.578	57–94
Poststep 1 outlier removal (FIGURE 1B)	104	0.836; 0.698	54–93
Poststep 2 outlier removal (FIGURE 1C)	97	0.877; 0.769	53–93
Final selected TRR			50–90[b]

a See FIGURE 1

b Rounded for ease of use by clinical/pharmacy/nursing staff. This TRR represents a calculated heparin range of 0.27–0.67 U/ml, and so remains essentially equivalent to 0.3–0.7 U/ml with rounding.

Abbreviations: see TABLES 1 and 2

and typically blood recollection is the only feasible option. Notably, even topping up underfilled lithium-heparin blood collection tubes with blood from a blood tube containing ethylenediaminetetraacetic acid (EDTA) dipotassium salt dihydrate may generate unreliable test results for numerous parameters, such as calcium, magnesium, potassium, chloride, and lactate dehydrogenase.[31]

In order to prevent carrying over of anticoagulants between blood collection tubes, a so-called "order of draw" has been proposed,[32] according to which the following sequence of blood collection tubes is specified: blood culture tubes, sodium citrate tubes, serum tubes (with or without a gel separator and/or a clot activator), heparin tubes (with or without gel), EDTA tubes, and tubes containing specific glycolysis inhibitors. This prevents crosscontamination with chemicals from different tubes, and helps avoid biased test results.

Conclusions Heparin use in the hospital system is ubiquitous. Accordingly, its presence in a sample tested in a hemostasis laboratory may be intended in the case of heparin therapy monitoring, or may be an unwanted interference in specific hemostasis assays. This review provides some guidance on optimizing the former, and minimizing the latter. At all times, good communication between clinicians requesting the assays and laboratories reporting the tests to the requesting clinicians provides the best outcomes.

ARTICLE INFORMATION

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REFERENCES

1 Fan BE, Favaloro EJ. Counting the carbon cost of heparin: an evolving tragedy of the commons? Lancet Haematol. 2022; 9: e469-e471.

2 Hirsh J, Warkentin TE, Shaughnessy SG. Heparin and low-molecular-weight heparin. Mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy and safety. Chest. 2001; 119: 64-94.

3 Safani M, Appleby S, Chiu R, et al. Application of anti-Xa assay in monitoring unfractionated heparin therapy in contemporary antithrombotic management. Expert Rev Hematol. 2023; 16: 1-8.

4 Favaloro EJ, Pasalic L. Lupus anticoagulant testing during anticoagulation, including direct oral anticoagulants. Res Pract Thromb Haemost. 2022; 6: e12676.

5 Stevens SM, Woller SC, Kreuziger LB, et al. Antithrombotic therapy for VTE disease: second update of the CHEST guideline and expert panel report. Chest. 2021; 160: e545-e608.

6 Dreijer AR, Diepstraten J, Leebeek FWG, et al. The effect of hospital-based antithrombotic stewardship on adherence to anticoagulant guidelines. Int J Clin Pharm. 2019; 41: 691-699.

7 Carpenè G, Negrini D, Lippi G, et al. Heparin: the journey from parenteral agent to nasal delivery. Semin Thromb Hemost. 2022; 48: 949-954.

8 Zhang J, Chen Z, Wang D, et al. Bivalirudin versus heparin in contemporary percutaneous coronary interventions for patients with acute coronary

syndrome: a systematic review and meta-analysis. Cardiol J. 2023 Nov 15. [Epub ahead of print]

9 Favaloro EJ, Kershaw G, Mohammed S, Lippi G. How to optimize activated partial thromboplastin time (APTT) testing: solutions to establishing and verifying normal reference intervals and assessing APTT reagents for sensitivity to heparin, lupus anticoagulant, and clotting factors. Semin Thromb Hemost. 2019; 45: 22-35.

10 Baluwala I, Favaloro EJ, Pasalic L. Therapeutic monitoring of unfractionated heparin - trials and tribulations. Expert Rev Hematol. 2017; 10: 595-605.

11 Marlar RA, Clement B, Gausman J. Activated partial thromboplastin time monitoring of unfractionated heparin therapy: issues and recommendations. Semin Thromb Hemost. 2017; 43: 253-260.

12 Dean CL. An overview of heparin monitoring with the anti-Xa assay. Methods Mol Biol. 2023; 2663: 343-353.

13 Adlard K, Brown C, Hayward S, et al. Pilot randomized trial of a three times weekly heparin flushing intervention in children, adolescents, and young adults with cancer with tunneled central venous catheters. J Pediatr Hematol Oncol Nurs. 2023; 40: 24-33.

14 López-Briz E, Ruiz Garcia V, Cabello JB, et al. Heparin versus 0.9% sodium chloride locking for prevention of occlusion in central venous catheters in adults. Cochrane Database Syst Rev. 2022; 7: CD008462.

15 Egnatios D, Gloria C. Implanted port patency: comparing heparin and normal saline. Clin J Oncol Nurs. 2021; 25: 169-173.

16 Moura EIM, de Brito GA, Alves JA, et al. Efficacy and safety of regional anticoagulation with 4% trisodium citrate versus heparin in extended hemodialysis among critical patients with cancer and acute kidney injury. Blood Purif. 2021; 50: 50-56.

17 Tang S, Xu L, Li H, et al. Anticoagulants in adult extracorporeal membrane oxygenation: alternatives to standardized anticoagulation with unfractionated heparin. Eur J Clin Pharmacol. 2023; 79: 1583-1594.

18 Rajsic S, Breitkopf R, Treml B, et al. Association of aPTT-guided anticoagulation monitoring with thromboembolic events in patients receiving V-A ECMO support: a systematic review and meta-analysis. J Clin Med. 2023; 12: 3224.

19 Schulman S, Sholzberg M, Spyropoulos AC, et al; International Society on Thrombosis and Haemostasis. ISTH guidelines for antithrombotic treatment in COVID-19. J Thromb Haemost. 2022; 20: 2214-2225.

20 Flisiak R, Horban A, Jaroszewicz J, et al. Management of SARS-CoV-2 infection: recommendations of the Polish Association of Epidemiologists and Infectiologists as of February 23, 2022. Pol Arch Intern Med. 2022; 132: 16230.

21 Keppel MH, Auer S, Lippi G, et al. Heparin and citrate additive carryover during blood collection. Clin Chem Lab Med. 2019; 57: 1888-1896.

22 Levy JH, Connors JM. Heparin resistance - clinical perspectives and management strategies. N Engl J Med. 2021; 385: 826-832.

23 Hardy M, Cabo J, Deliège A, et al. Reassessment of dextran sulfate in anti-Xa assay for unfractionated heparin laboratory monitoring. Res Pract Thromb Haemost. 2023; 7: 102257.

24 Lasne D, Toussaint-Hacquard M, Delassasseigne C, et al. Factors influencing anti-Xa assays: a multicenter prospective study in critically ill and noncritically ill patients receiving unfractionated heparin. Thromb Haemost. 2023; 123: 1105-1115.

25 Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost. 2018; 118: 437-450.

26 Douxfils J, Adcock D, Bates SM, et al. 2021 Update of the International Council for Standardization in Haematology recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost. 2021; 121: 1008-1020.

27 Marlar RA, Gausman JN. The effect of instrumentation and laboratory site on the accuracy of the APTT-based heparin therapeutic range. Int J Lab Hematol. 2012; 34: 614-620.

28 Marlar RA, Gausman J. The optimum number and types of plasma samples necessary for an accurate activated partial thromboplastin time-based heparin therapeutic range. Arch Pathol Lab Med. 2013; 137: 77-82.

29 Favaloro EJ, Mohammed S, Vong R, et al. Verification of the ACL Top 50 family (350, 550 and 750) for harmonization of routine coagulation assays in a large network of 60 laboratories. Am J Clin Pathol. 2021; 156: 661-678.

30 Heparin Resistant Recalcifying Solution. https://www.haematex.com/haematex-products/antihepca-hrrs. Accessed December 24, 2023.

31 Lima-Oliveira G, Salvagno GL, Danese E, et al. Contamination of lithium heparin blood by K2-ethylenediaminetetraacetic acid (EDTA): an experimental evaluation. Biochem Med (Zagreb). 2014; 24: 359-367.

32 Cornes M, van Dongen-Lases E, Grankvist K, et al; Working Group for Preanalytical Phase (WG-PRE), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Order of blood draw: opinion paper by the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE). Clin Chem Lab Med. 2017; 55: 27-31.