DR STEVEN PIPE (Orcid ID : 0000-0003-2558-2089)
DR SHIH-HON LI (Orcid ID : 0000-0003-4538-8339)

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Anti-Factor IIa (FIIa) Heparin Assay for Patients on Direct Factor Xa (FXa) Inhibitors

Morgan Stuart,* Linda Johnson,* Sarah Hanigan,† Steven W. Pipe,*‡ and Shih-Hon Li*
*Special Coagulation Laboratory, Department of Pathology; †College of Pharmacy; and ‡Department of Pediatrics, University of Michigan Medical School, Ann Arbor, MI, USA

Correspondence:
Shih-Hon Li
Department of Pathology
University of Michigan Medical School
1301 Catherine St, Ann Arbor, MI 48109
USA
Email: shihhon@med.umich.edu

Essentials:

- Therapeutic direct factor Xa (FXa) inhibitors interfere with anti-FXa unfractionated heparin (UFH) assays.
- An UFH assay based on inhibition of thrombin (FIIa) was evaluated for clinical use.
- The anti-FIIa UFH assay was free from interference by direct FXa inhibitors, but also increased reagent costs.
- The anti-FIIa assay led to fewer critically high UFH results than the anti-FXa assay for patients transitioning a direct FXa inhibitor to UFH.

Summary:

Background: Direct factor Xa (FXa) inhibitors are increasingly prescribed for outpatients, and those transitioning to unfractionated heparin (UFH) for hospital admission are monitored via an anti-FXa assay. Due to assay interference, UFH results would often be critically elevated, confounding dosing.

Objectives: An anti-factor IIa (FIIa) UFH assay was evaluated for clinical use.

Methods: The BIOPHEN™ ANTI-IIa (Aniara Diagnostica) assay and anti-FXa INNOVANCE® Heparin assay (Siemens Healthcare Diagnostics Products GmbH) were compared on the Siemens BCS XP system. Samples included UFH controls and calibrators and specimens from patients transitioning from apixaban or rivaroxaban to UFH. Method comparison, linearity, recovery, precision, and interference by direct FXa inhibitors were evaluated. The effect of the BIOPHEN™ ANTI-IIa assay on the rate of critically high UFH results was retrospectively reviewed 4 months after implementation.

Results: Accuracy studies using 0.24 and 0.50 IU/mL UFH yielded means and standard deviations of 0.26 ± 0.01 and 0.58 ± 0.01 IU/mL, respectively. Within-run and between-run coefficients of variation were 4.6% and 15.5% for the low control, and 1.8% and 10.6% for the high control. The method comparison slope was 0.9965 (r^2 = 0.9468). The linear range was 0.1 – 1.3 IU/mL. The assay measured UFH in the presence of 192 ng/mL apixaban or 158 ng/mL rivaroxaban. Introduction of the assay for clinical use reduced the monthly percentage of critically high results from 9.4% to 3.8% for admitted heparinized patients who recently discontinued apixaban or rivaroxaban.

Conclusions: The BIOPHEN™ ANTI-IIa assay is suitable for patients transitioning off apixaban or rivaroxaban.

Keywords:

- Apixaban
- Drug monitoring
- Factor IIa
- Heparin
- Rivaroxaban

Introduction:

Oral direct factor Xa (FXa) inhibitors, such as rivaroxaban, apixaban, and edoxaban, are widely used in the management of venous thromboembolism and atrial fibrillation (AF) [1]. They selectively bind and inactivate the active sites of free and prothrombinase-complexed FXa, thereby decreasing thrombin generation. Direct FXa inhibitors are primarily excreted in urine and feces and exhibit terminal half-lives of 5 to 15 hours, depending on the inhibitor [2]. Due to these predictable pharmacokinetic properties, their use does not require drug level monitoring, belying their growing popularity. However, their presence in patient plasma specimens can cause interferences in numerous clinical coagulation assays, including prothrombin time coagulation factor assays, activated partial thromboplastin time coagulation factor assays, and lupus anticoagulant assays [3,4].

Often, patients on direct FXa inhibitors are transitioned to shorter acting anticoagulants such as unfractionated heparin (UFH) when they are admitted to the hospital for invasive procedures or for new thrombosis. Within our health system, UFH levels in such patients are monitored via an anti-FXa assay with UFH calibrators. The risk of interference by direct FXa inhibitors in this assay is high since their anti-FXa activities may be transformed into falsely increased UFH results [5]. Indeed, Macedo, *et al*, demonstrated the amount of critically high UFH results in inpatient units frequently transitioning patients from direct FXa inhibitors to UFH was higher than that of the overall hospital. Furthermore, such critically high results led to decreasing or even discontinuing UFH and triggered increased anti-FXa monitoring with negative implications on cost and patient convenience. They also opined that holding or reducing an UFH infusion because of an elevated anti-FXa level related to a direct FXa inhibitor may also pose undue harm to patients if this occurred in the context of an acute thrombotic event [5]. Thus, a different management strategy was necessitated.

The current study evaluates an assay for the quantitation of UFH based on the inhibition of thrombin (FIIa) rather than on FXa. The feasibility of adapting such an assay for a popular commercial automated clinical coagulation analyzer is demonstrated and its performance investigated with regard to linearity and recovery, measurement range, accuracy, precision, and interference by the direct FXa inhibitors on the University of Michigan pharmacy formulary, rivaroxaban and apixaban. Via retrospective test utilization review, the effect of such an assay on the rate of critically high UFH in a selected population is quantified. Lastly, the implementation of the anti-FIIa UFH assay for a select patient population and alternatives for a spectrum of clinical coagulation laboratory practices are discussed.

Methods:

The BIOPHEN™ ANTI-IIa (2 Stages Heparin Assay) kit and UFH calibrators and controls were purchased from Aniara Diagnostica LLC (West Chester, OH). BIOPHEN™ apixaban and rivaroxaban calibrators and controls were also from Aniara Diagnostica. The purity of raw apixaban and rivaroxaban materials used in the creation of the calibrators and controls were >99% per the manufacturer. The anti-FXa INNOVANCE® Heparin Assay, UFH calibrators, and UFH controls were from Siemens Healthcare Diagnostics Products GmbH (Marburg, Germany). CRYOcheck™ pooled normal plasma (NPP) was from Precision Biologic, Inc (Dartmouth, NS, Canada). UFH linearity samples were purchased from the College of American Pathologists (Northfield, IL). Fifty-four residual, post-test patient plasma specimens that were routinely submitted for anti-FXa UFH testing in blue-top BD Vacutainer® tubes with 3.2% sodium citrate (Becton, Dickinson and Company, Franklin Lakes, NJ) were collected and de-identified for the study. The study met criteria for Not Regulated Human Research as set forth by the Institutional Review Board of the University of Michigan Medical School, Ann Arbor.

The INNOVANCE® Heparin assay is cleared by the United States Food and Drug Administration for clinical testing and was performed per manufacturer's instructions on the BCS XP system (Siemens Healthcare Diagnostics Products) without modification [6]. Despite being designed for a Stago coagulation analyzer and manual testing, the BIOPHEN™ ANTI-IIa kit was installed for use on a Siemens BCS XP system using the package insert and application guide to develop the assay program [7,8]. The provided BIOPHEN™ ANTI-IIa assay synopsis was: sample diluted 1:40 in buffer, addition of R1 (reagent containing human antithrombin), addition of R2 (reagent containing human thrombin) followed by a 120 second incubation at 37 °C, addition of R3 (reagent containing a thrombin-specific chromogenic substrate), measuring the OD/min change in colorimetric signal for 15 – 40 seconds at 405 nm, and calculating the UFH level using a Lin-Log calibration curve [7,8]. To adapt this for the Siemens BCS XP system, incubation times and volumes were modified while keeping reagent and sample ratios constant. A Lin-Lin calibration curve and a saline solution enzyme blank were also added. The programming was judged as successful when the calibration curve was performed without any flags and the BIOPHEN™ Unfractionated Heparin quality control (UFH QC) values were within the manufacturer's defined limits. The successful BCS XP system programming is summarized in Figure S1. All anti-FIIa UFH levels for this study were measured using the BIOPHEN™ ANTI-IIa kit on the Siemens BCS XP system.

Method comparison between the anti-FXa INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay was performed using a combination of sixteen UFH calibrator dilution samples, twelve UFH linearity samples, and fourteen consecutive residual post-test samples from patients known to be anticoagulated only with UFH. Within-run precision of the BIOPHEN™ ANTI-IIa assay was performed by measuring the BIOPHEN™ UFH control levels 1 and 2 (0.24 IU/mL and 0.50 IU/mL, respectively [9]) 20 consecutive times. Between-run precision was performed by measuring the BIOPHEN™ UFH control materials 1 – 6 times per day for 25 days (n = 82). Linearity was performed by dilution of the INNOVANCE® Heparin calibrators and BIOPHEN™ UFH controls into NPP and then measurement via the BIOPHEN™ ANTI-IIa assay. Recovery was assessed by dividing the measured linearity results by the expected UFH levels.

For *in vitro* interference studies, BIOPHEN™ apixaban and rivaroxaban Calibrator 2 (Cal2) and Control 2 (C2) were reconstituted per manufacturer's instructions by adding 1 mL NERL™ Reagent Grade Water (Thermo Fisher Scientific, Waltham, MA), shaking vigorously until complete dissolution, allowing to stabilize at room temperature for 30 minutes, and homogenizing before use. Per the Certificates of Analysis, reconstituted concentrations were 384 ng/mL for apixaban C2 (lot F1700657/F1700657), 290 ng/mL for apixaban Cal2 (lot F1700659/F1700660), 316 ng/mL for rivaroxaban C2 (lot F1600888), and 250 ng/mL for rivaroxban Cal2 (lot F1700285/F1700286). While reconstituted controls are stable for 7 days at

2 – 8 °C, vials prepared for the interference assays were used within 8 hours. The sensitivities of the INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay to direct FXa inhibitors were evaluated with dilutions of apixaban and rivaroxaban Cal2 in NPP in the absence of UFH. To test the assays' ability to measure UFH in the presence of direct FXa inhibitors, samples with a range of UFH levels were assembled by mixing different amounts of BIOPHEN™ UFH Controls 1 and 2 with each other without diluent. Then, each of these UFH samples were combined 1:1 with equal volumes of reconstituted apixaban or rivaroxaban C2, and analyzed using the INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay.

The stability of the BIOPHEN™ reagents including UFH controls was monitored hourly at room temperature for 24 hours without re-refrigeration. Calibration curve stability was indirectly monitored after BIOPHEN™ ANTI-IIa assay implementation by reviewing results of routine quality control testing.

The rate of critically high UFH results was retrospectively determined. The inpatient units from which the BIOPHEN™ ANTI-IIa assay was most ordered were identified using the laboratory's information system, SCC Soft Computer SoftLab (Clearwater, FL) and results from the first 4 months of testing after implementation collected. From those same inpatient units, four months of results from the INNOVANCE® Heparin assay immediately preceding implementation of the BIOPHEN™ ANTI-IIa assay were also collected. From this pre-implementation dataset, INNOVANCE® Heparin assay results from patients transitioning onto UFH from apixaban or rivaroxaban were isolated via manual review using the Electronic Medical Record Search Engine (EMERSE) available at University of Michigan [10].

Data were analyzed using GraphPad Prism 8.3.0 (San Diego, CA). Statistical significance was determined using a non-parametric, two-tailed Mann-Whitney U test.

Results:

Method comparison

Forty-two samples free from direct FXa inhibitors (14 remnant patient specimens, 12 proficiency testing linearity samples, and 16 UFH Calibrator dilutions) were tested using the INNOVANCE® Heparin assay and BIOPHEN™ ANTI-IIa assay. Linear regression analysis of results from the two assays showed a slope of 0.9965 (r^2 = 0.9468) and a correlation coefficient, r, of 0.9730

(Figure 1A). Relative bias was consistently within 20% by Bland-Altman analysis except when average results were below 0.1 IU/mL UFH (Figure 1B).

Precision

The BIOPHEN™ UFH control level 1 has an expected UFH concentration of 0.24 IU/mL [9]. Within-run precision showed a mean of 0.26 IU/mL, with a SD of 0.01 IU/mL, and a CV of 4.6%. Between-run precision showed a mean of 0.20 IU/mL, with a SD of 0.03 IU/mL, and a CV of 15.5%. The BIOPHEN™ UFH control level 2 has an expected UFH concentration of 0.50 IU/mL [9]. Within-run precision showed a mean of 0.58 IU/mL, with a SD of 0.01 IU/mL, and a CV of 1.8%. Between-run precision showed a mean of 0.52 IU/mL, with a SD of 0.05 IU/mL, and a CV of 10.6%. Manufacturer expected within-run CVs for UFH control levels 1 and 2 were 6.6% and 4.1% [7], respectively, while the maximally acceptable between-run CV is 20% for University of Michigan Department of Pathology clinical laboratories.

Linearity and recovery

Using INNOVANCE® Heparin calibrator samples and BIOPHEN™ UFH control samples, thirty-six samples of known UFH concentration were created via dilution into NPP and analyzed by the BIOPHEN™ ANTI-IIa assay. Linear regression analysis of measured anti-FIIa UFH results versus expected UFH concentration showed a slope of 0.9640 (R^2 = 0.9631; Figure 2A) with responses again becoming non-linear below 0.1 IU/mL (Figure 2B). Recovery was 111 ± 20% across the concentrations tested. Based on the method comparison (Figure 1) and linearity results (Figure 2), the measurable UFH range for the BIOPHEN™ ANTI-IIa assay was set at 0.1 – 1.3 IU/mL.

Interference

The sensitivities of the INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay to direct FXa inhibitors were evaluated using doses of apixaban and rivaroxaban in NPP without UFH. The INNOVANCE® Heparin assay reported the anti-FXa activities of the inhibitors as UFH levels across its reportable range (Figure 3A). However, the BIOPHEN™ ANTI-IIa assay was insensitive to apixaban and rivaroxaban up to 400 ng/mL, reporting out no detectable UFH levels. To evaluate the performance of the assays in mixtures of UFH and direct FXa inhibitor, a range of UFH concentrations were created by mixing BIOPHEN™ UFH control plasmas. Then, these were combined with apixaban (final concentration 192 ng/mL) or rivaroxaban (final concentration 158 mg/mL) and analyzed. The BIOPHEN™ ANTI-IIa assay yielded reportable results compatible with the expected UFH concentrations (Figure 3B). In contrast, by the INNOVANCE® Heparin assay, all samples were resulted as >1.5 IU/mL, consistent with the interfering effect of apixaban and rivaroxaban in the anti-FXa UFH assay shown in Figure 3A.

Assay stability

All BIOPHEN™ ANTI-IIa assay reagents were left on board the BCS XP system at room temperature, and the BIOPHEN™ UFH control levels 1 and 2 were run once every hour for 24 hours. For the first 12 hours, the CVs were 5.6% and 16.5%, respectively (Figure 4). However, for the second 12 hours, the CVs worsened to 9.4% and 24.6%, respectively (Figure 4), indicating reagent instability. To address this problem, we implemented the procedure of storing all BIOPHEN™ ANTI-IIa reagents at 2 – 8 °C, heating R1, R2, and R3 to 37 °C by placing on a heat block for 15 minutes just before use to ensure proper heat activation of the reagents, and analyzing the UFH control levels immediately before analyzing patient specimens. The BIOPHEN™ and INNOVANCE® quality control aliquots are each used for up to 48 hours. The INNOVANCE® controls are run once every 8 hours of patient testing or at each reagent vial change, while the BIOPHEN™ controls are run before every patient run. This belies the increased reagent cost per test of the BIOPHEN™ ANTI-IIa assay ($3.83 USD) as compared to the INNOVANCE® Heparin assay ($2.86 USD).

The BIOPHEN™ ANTI-IIa assay calibration curve appears to be as stable as that for the INNOVANCE® Heparin assay. In the year since it was implemented, the BIOPHEN™ ANTI-IIa assay was calibrated every 6 months as scheduled, once when there was an assay kit lot change, and once when there was an unusual trend in quality control results. This was identical to anti-FXa INNOVANCE Heparin assay in the past year, which also required one recalibration due to an unusual quality control result trend. Therefore, calibrator consumption is anticipated to be comparable between the two assays.

Effect on critically high results

The performance of the anti-FXa INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay on specimens from patients transitioning from anticoagulation with direct FXa inhibitors to UFH were evaluated. Residual samples from 40 consecutive plasma specimens collected in the Emergency Department (ED) for routine UFH levels were tested by both assays. These specimens were from patients who were receiving outpatient treatment with apixaban or rixaroxaban, and were transitioned on UFH per anticoagulation guidelines for atrial fibrillation or acute coronary syndrome (AF/ACS). In all cases, the INNOVANCE® Heparin assay reported higher UFH results than the BIOPHEN™ ANTI-IIa assay (Figure 5). Furthermore, 22 of the 40 samples were reported to have critically high UFH levels (>1.0 IU/mL) by the INNOVANCE® Heparin assay, with 13 of those results being above the reportable range (resulted as >1.5 IU/mL but plotted as 1.5 IU/mL). In contrast, by the BIOPHEN™ ANTI-IIa assay, no results were above the reportable range, and only 2 of the 40 samples were reported to have critically high UFH levels. These two high anti-FIIa UFH results appeared to be truly due to supratherapeutic plasma UFH concentrations, since contemporaneous activated partial thromboplastin times (aPTT) results were also critically prolonged beyond the reportable range (>120 s) for both patients and neither had been given other potentially interfering anticoagulants such as argatroban, dabigatran, or enoxaparin.

The effect of the BIOPHEN™ ANTI-IIa assay on critically high UFH results was retrospectively studied as described for patients transitioning from direct FXa inhibitors to UFH. Approximately 75% of BIOPHEN™ ANTI-IIa assay orders were from 6 inpatient clinical areas: 4 cardiology units, 1 medical acute care unit, and 1 medical/surgical moderate care unit. The UFH results from patients in these units were analyzed for 4 months immediately prior to and immediately following implementation of the BIOPHEN™ ANTI-IIa assay for clinical use. Common indications for outpatient use of apixaban or rivaroxaban included atrial fibrillation and history of deep venous thrombosis and/or pulmonary embolism. Common indications for inpatient transition to UFH included ACS, cardioversion, and coronary artery catheterization, as well as unrelated comorbidities and procedures/surgeries. Pre-implementation, only the anti-FXa INNOVANCE® Heparin assay was available to monitor UFH and yielded a monthly percentage of critically high results of 9.4% (6.2 – 14.2%) (median, 25^{th} – 75th percentile) (Figure 6). Post-implementation, patients with recent apixaban or rivaroxaban use were first monitored via the BIOPHEN™ ANTI-IIa assay and then the INNOVANCE® Heparin assay, yielding a significantly decreased percentage of critically high results at 3.8% (3.4 – 4.9%) (n = 4, P = 0.0286). The percentage of critically high UFH results from the BIOPHEN™ ANTI-IIa assay alone was even lower at 1.3% (0.3 – 2.9%) (P = 0.0286, compared to pre-implementation percentages). These findings imply that the BIOPHEN™ ANTI-IIa assay can prevent falsely high UFH results in real-world clinical settings.

<u>Discussion</u>:

The inability to appropriately adjust UFH dosing in patients transitioning from direct FXa inhibitors to UFH compromises the therapeutic efficacy of UFH, the safety of patients, and the ability of hospitals to provide high quality patient care. Our approach to addressing this problem involves a close collaboration between the Departments of Pathology and Pharmacy. It was agreed that the solution should be straightforward for the laboratory and compatible with the existing dosing nomogram based on peripheral blood UFH levels. Validation of an anti-FIIa UFH assay as a laboratory derived test met those criteria. To avoid overutilization because of its increased cost and slightly more complex workflow, the BIOPHEN™ ANTI-IIa assay is a restricted test. Pharmacy colleagues remain instrumental in identifying appropriate patients for the BIOPHEN™ ANTI-IIa assay as well as in determining the appropriate time at which a patient's UFH levels can be safely monitored by the anti-FXa INNOVANCE® Heparin assay after clearance of any direct FXa inhibitors. We now also leverage the electronic medical record to alert providers ordering the UFH nomogram whether their patients have recent direct FXa inhibitor use and to direct providers to the appropriate laboratory testing and UFH monitoring strategy.

There are alternative laboratory solutions that avoid interference in UFH monitoring by direct FXa inhibitors. Before testing, patient plasma specimens can be pretreated with activated charcoal products, DOAC-Stop (Australian Scientific Enterprise, Hornsby, NSW) [11] or DOAC-Remove (5-Diagnostics AG, Basel, Switzerland), which can remove several classes of direct oral anticoagulants. DOAC-Stop is reported to completely remove 1000 ng/mL apixaban or 600 ng/mL rivaroxaban in NPP, while in separate samples, having no effect on 200 ng/mL UFH or 4000 ng/mL enoxaparin [11]. However, pretreatment with activated charcoal would have added an additional manual step to the laboratory workflow and would contribute to additional cost added to the assay itself. Thrombin time was also considered, since it is also not affected by direct FXa inhibitors. However, this would have required the creation and validation of an entirely new UFH dosing nomogram based on thrombin time, as well as training of clinical staff to use the new nomogram in addition to the pre-existing one based on measured UFH level. While these alternatives did not meet the needs of our laboratory users, they may be more appropriate options than the BIOPHEN™ ANTI-IIa assay for other institutions after workflow, cost, and clinical experiences are weighed.

There are important limitations to the BIOPHEN™ ANTI-IIa assay. First, while it is insensitive to direct FXa inhibitors, based on its methodology, the assay is very likely susceptible to interference by other anticoagulants, such as enoxaparin, fondaparinux, and dabigatran. Such analytical error is best mitigated via acquisition of accurate medication histories and judicious use of the assay only for patients treated with direct FXa inhibitors and initiating UFH therapy. In cases where clinical information may be unavailable, such as in unresponsive patients, we would use the anti-FXa INNOVANCE® Heparin assay having no indication to use the BIOPHEN™ ANTI-IIa assay. If anti-FXa UFH levels are unexpectedly high, we would correlate with aPTT and anti-FIIa UFH results, as well as continue efforts to gather relevant clinical history. Secondly, the addition of exogenous human antithrombin may cause the BIOPHEN™ ANTI-IIa assay to overestimate the biological effect of UFH in patients with antithrombin deficiency and lead to sub-therapeutic anticoagulation. While the frequency of hereditary antithrombin deficiency is about 1 in 2000 – 3000 individuals, acquired antithrombin deficiency is associated with liver disease, malnutrition, nephrotic syndrome, hemodialysis, sepsis, and major surgery and trauma [12]. Anti-FXa INNOVANCE® Heparin assay results that are incongruently low compared to UFH dosing may indicate the presence of underlying antithrombin deficiency; however, in the presence of direct FXa inhibitors, that scenario would be unlikely. Therefore, neither assay is ideal for antithrombin deficient patients with both direct FXa inhibitors and UFH in their circulation. In our clinical practice, we aim to promptly discontinue use of the BIOPHEN™ ANTI-IIa assay and convert patients to the INNOVANCE® Heparin assay after the expected clearance of direct FXa inhibitors, which would somewhat mitigate assay inflation of the biological UFH effect. If antithrombin deficiency is suspected and needs to be ruled out but direct FXa inhibitor effect is still present, FIIa-based antithrombin activity assays or immunologic antigen assays would be preferred over FXa-based activity assays.

There were also limitations to this study. First, the validation relied on spiked UFH samples to supplement residual patient specimens in order to interrogate the entire clinically relevant range of the UFH assays. De-identified post-test residual patient samples generally provided UFH levels in the low end of the therapeutic range. Purchased UFH linearity samples and reagent plasma spiked with UFH calibrator dilutions were required to evaluate the assays in the high end of the therapeutic range and the supra-therapeutic range. Consequently, UFH sources and specimen matrices were non-identical. Interference experiments were also subject to a similar limitation. UFH and direct FXa inhibitor levels cannot be determined in residual specimens from patients on both types of anticoagulant due to mutual assay interference. Thus, samples with known amounts of UFH and apixaban or rivaroxaban had to be created in the laboratory to assess the performance of the BIOPHEN™ ANTI-IIa and INNOVANCE® Heparin assays in the presence of direct FXa inhibitors. A second limitation was that all patient specimens used for this validation study were from adults, since anti-FIIa assays are reported to be less responsive than anti-FXa assays to UFH in pediatric patients [13]. Therefore, the validated BIOPHEN™ ANTI-IIa assay is only available for adult patients at University of Michigan.

As direct FXa inhibitors continue to gain popularity, current approaches to patient UFH monitoring will need to be adjusted or augmented to reduce interference and delay to reaching appropriate dosing of UFH. Here we have shown that for a targeted patient population, a test method based on the anti-FIIa activity of UFH is a viable solution. The BIOPHEN™ ANTI-IIa assay is able to provide actionable UFH results in the presence of direct FXa inhibitors and should be considered for addition to the anticoagulation management toolbox of clinical coagulation laboratories.

Addendum: S. Hanigan, S. W. Pipe, and S. H. Li conceived and designed the study and analysis. M. Stuart and L. Johnson performed the experiments and collected the data. M. Stuart and S. H. Li performed the analysis and drafted the manuscript. M. Stuart, L. Johnson, S. Hanigan, S. W. Pipe, and S. H. Li revised the final manuscript.

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Figure 1. (A) Method comparison between the INNOVANCE® Heparin assay ("Anti-FXa UFH") and the BIOPHEN™ ANTI-IIa assay ("Anti-FIIa UFH") on 42 samples containing UFH as the only anticoagulant medication, including residual post-test patient specimens, commercial linearity samples, and UFH calibrator dilutions. The results were analyzed via linear regression

(*solid line*, slope = 0.9965, R^2 = 0.9468, solid line; *dashed line*, line of equality) and (B) Bland-Altman plot.

Figure 2. To determine the linear range, manufacturers' heparin control and calibrator materials were used to create 36 samples of known UFH concentration which were then tested by the BIOPHEN™ ANTI-IIa assay. The results were analyzed by (A) linear regression (*solid line*, slope = 0.9640, R^2 = 0.9631; *dashed line*, line of equality) and (B) relative residuals.

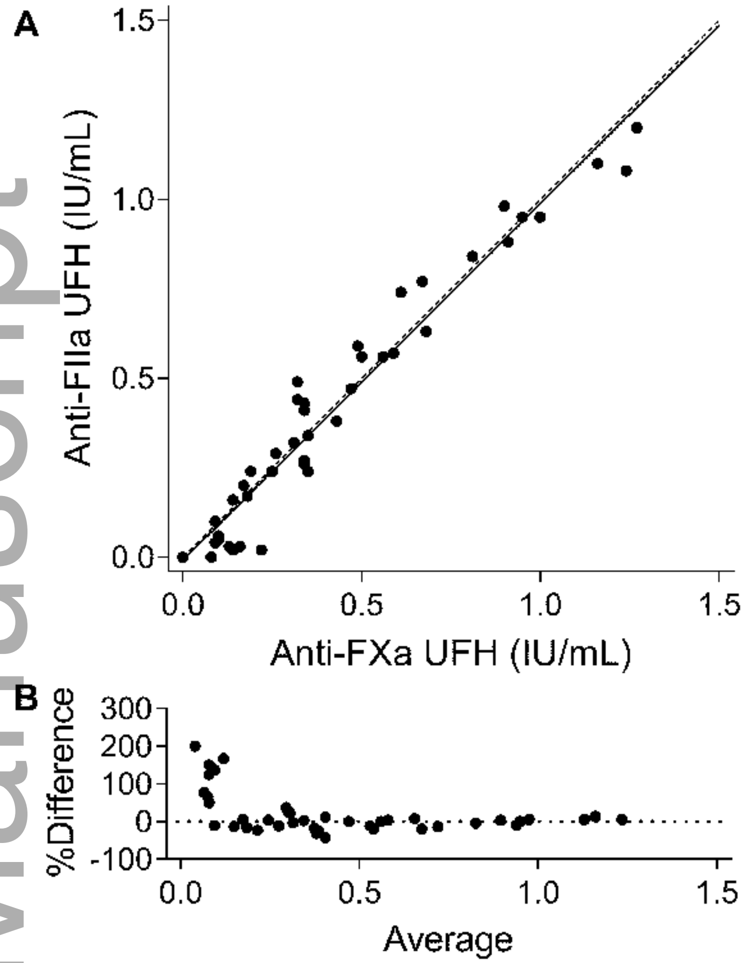
Figure 3. (A) Samples of apixaban (*closed triangles*) or rivaroxaban (*open circles*) in NPP without UFH were tested with the INNOVANCE® Heparin assay ("Measured Anti-FXa UFH") in triplicate. The BIOPHEN™ ANTI-IIa assay reported no detectable UFH levels for up to 400 ng/mL apixaban or rivaroxaban, as all samples were resulted as below the assay's reportable range. (B) A range of UFH concentrations with either 192 ng/mL apixaban (*closed triangles*) or 158 ng/mL rivaroxaban (*open circles*) were tested by the BIOPHEN™ ANTI-IIa assay ("Measured Anti-FIIa UFH"). When the same samples were tested using the INNOVANCE® Heparin assay, all results were >1.5 IU/mL, exceeding the reportable UFH range of the assay.

Figure 4. The stability of the BIOPHEN™ reagents and controls were evaluated by leaving them on board the BCS XP system at room temperature. Once per hour for 24 hours, UFH control levels 1 (*open circles*) and 2 (*closed triangles*) were tested using the BIOPHEN™ ANTI-IIa assay. The CV was calculated for each level of control for the first and second 12 hours.

Figure 5. Specimens from 40 patients on a direct FXa inhibitor who presented to the ED and were subsequently started on UFH per institutional AF/ACS anticoagulation guidelines were tested using the INNOVANCE® Heparin assay ("Anti-FXa") and the BIOPHEN™ ANTI-IIa assay ("Anti-FIIa"). The area in grey denotes the critically high range.

Figure 6. The monthly percentage of critically high UFH results for inpatient units frequently using the BIOPHEN™ ANTI-IIa assay for patients transitioning from a direct FXa inhibitor to UFH was analyzed for 4 months before and after implementation of the assay for clinical use. Pre-implementation UFH results were from the INNOVANCE® Heparin assay only, while post-implementation UFH results were from a combination of the INNOVANCE® Heparin assay and the BIOPHEN™ ANTI-IIa assay. The rates of critically high UFH results from the BIOPHEN™ ANTI-IIa assay ("Anti-FIIa UFH only") were also examined. Significance was analyzed using a

non-parametric, two-tailed Mann-Whitney U test (*, $P < 0.05$). Lines and whiskers represent medians and interquartile ranges.



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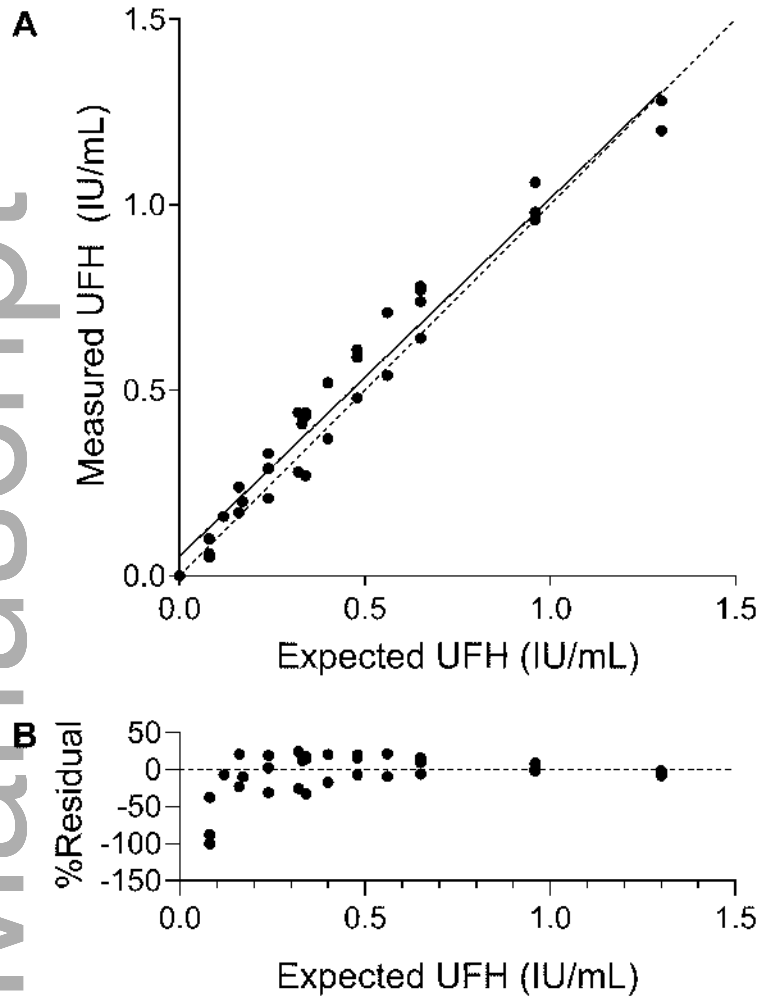

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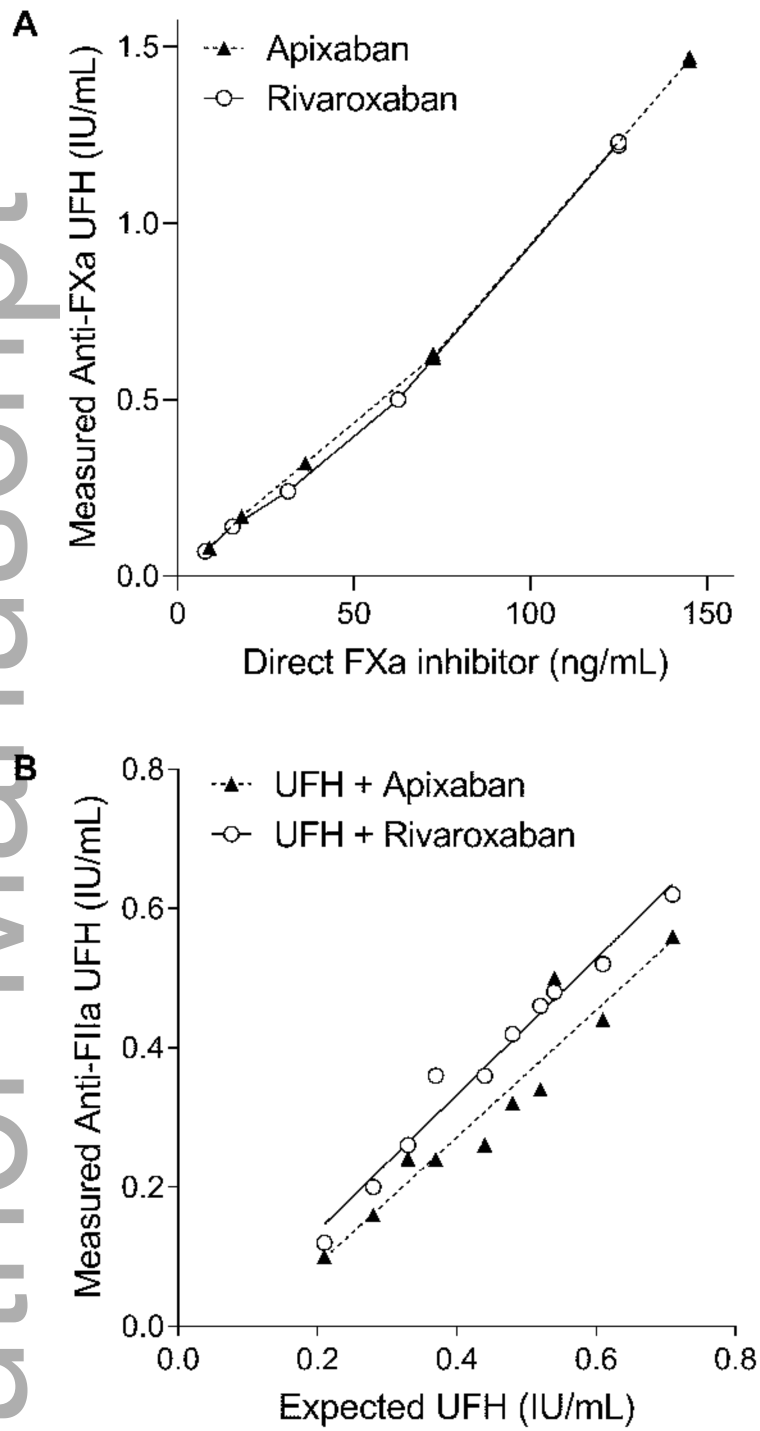

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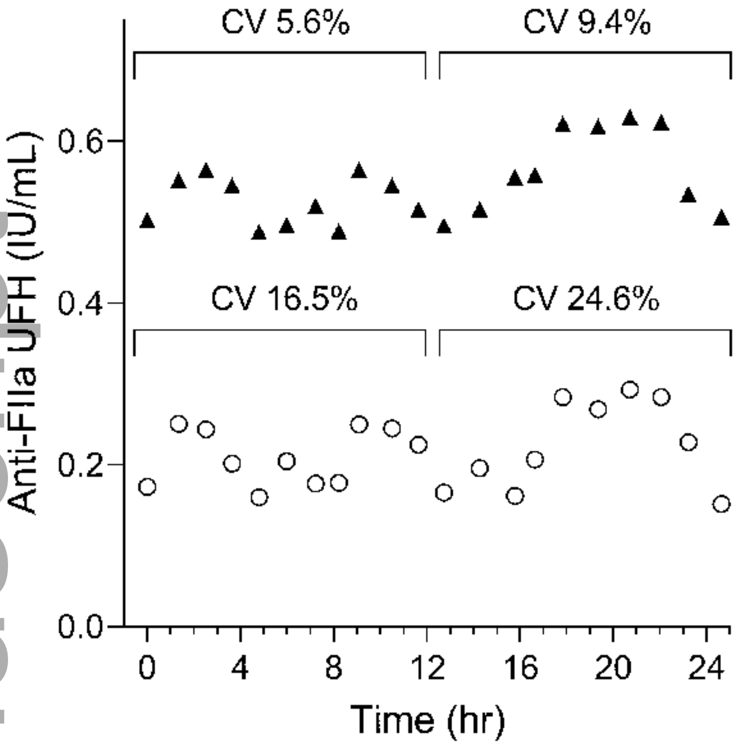

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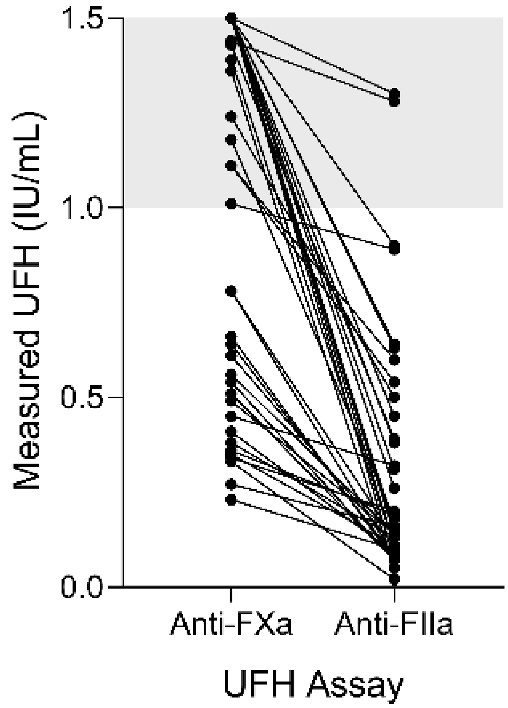

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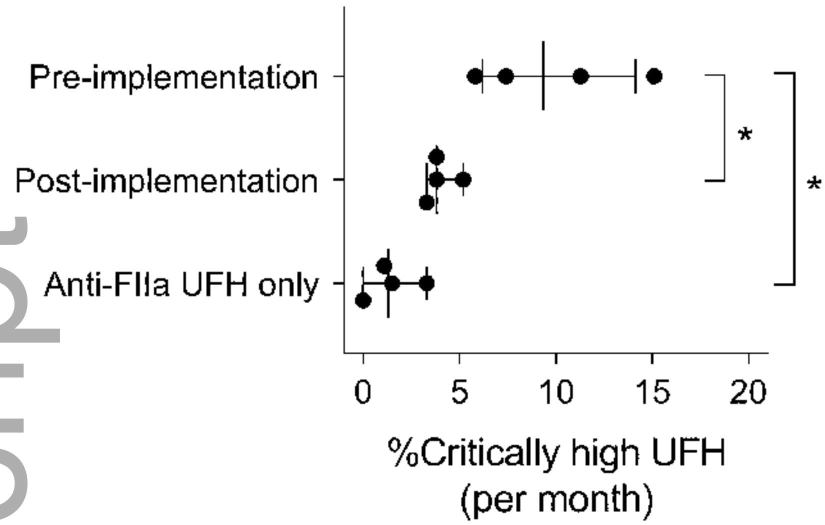

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