The effect of freezing and thawing of samples for anti-Factor Xa testing for the determination of enoxaparin activity

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Dear Sir,

The quality and validity of laboratory results, especially for coagulation analyses, are influenced by pre-analytical variables including the collection technique, transportation, pre-analytical processing and storage of samples.[1] Laboratory networks often rely on centralization of specialized testing, which necessitates on-site preparation of samples and transportation to referral centres. Low molecular weight heparin (LMWH) offers improved clinical predictability of anticoagulation and often does not require routine monitoring. Certain clinical scenarios, however, such as extremes of age, obesity and renal dysfunction, may influence anti-factor Xa (anti-FXa) levels, and in these circumstances, anti-FXa laboratory activity testing is indicated.[2] This test is not always available in smaller peripheral laboratories, and in these cases, samples must be centrifuged, separated and frozen on-site and then referred to the testing facility. Freezing is commonly used to stabilize analytes prior to referral especially for coagulation testing where analyte half-lives may be short but freezing may influence test results and the effects should be assessed in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[3, 4]

We conducted a study to assess the effect of a single freeze-thaw cycle on the results obtained for anti-FXa analysis. Ethics approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (M150160). Forty randomly selected samples, received for anti-FXa testing in citrate disodium tubes (Becton Dickinson®) within 4 hours of collection at the National Health Laboratory Service (NHLS) coagulation laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), were included (Table 1). This laboratory is a tertiary referral facility receiving samples for coagulation testing from across South Africa.

Two validated coagulation analytical platforms, widely utilized throughout the NHLS laboratory network, were selected. Prior to sample analysis, the analysers were calibrated with STA® Multi Hep Calibrator on the STA-R Max® (Diagnostica Stago®) and Innovance® Heparin Calibrator on the Sysmex® (Siemens®) analysers. Samples were centrifuged in order to obtain platelet poor plasma and then analysed on a Stago STA R Max® automated coagulation analyser using STA® Liquid Anti-FXa reagent after acceptable results were obtained with STA® Quality HBPM/LMWH control material and then frozen at −80°C. Samples were then transported frozen to sister laboratories at Chris Hani Baragwanath and Steve Biko Academic Hospital laboratories for analysis on Sysmex CS-2500i® automated analysers with Siemens Innovance® Heparin assay reagent after successful calibration with Innovance® Heparin Calibrator and acceptable results were obtained with Innovance® Heparin UF and LMW control material. The lower limit of detection for both reagents is stated as 0.1 IU/mL. Equivalence of these two analytical platforms and reproducibility of results on frozen samples have previously been verified in our laboratory as a component of analyser validation.[5, 6]

40 samples were tested before and after freezing and the anti-FXa study results were expressed in IU/mL.

Critical values were computed according to the American College of Chest Physicians (ACCP) reference range categories, which are as follows[7]:

a) <0.2 IU/mL – subtherapeutic anti-FXa level

b) 0.2-0.6 IU/mL – prophylactic anti-FXa level

c) >0.6 – ≤ 1.2 IU/mL –therapeutic anti-FXa level

The ability of the assay to assess accurately within these 3 levels was reported on the thawed samples, and sensitivity, specificity and related statistics were calculated for each reference range (Table 1).

Table 1. A comparison of the descriptive statistics between the initial anti-FXa analysis on fresh plasma (at CMJAH) and the analysis post-thaw (at Chris Hani and Steve Biko Academic Hospitals)

	Post-thaw	Initial
Mean	0.46	0.45
Variance	0.12	0.13
Observations	36	36
Correlation (overall)	0.9923	
P-value (overall)	<.001	
Regression line = 0.9612 95% CI [0.9195; 1.0029]		
Regression line *p*-value	<.001	
Regression model fit R^2	.9847	

Sensitivity analysis	Reference range categorie (IU/mL)		
	0-0.2	>0.2-0.6	>0.6 to <1.2
	n = 11	n=14	n=10
Sensitivity	100%	100%	100%
Specificity	100%	100%	100%
False positives	0%	0%	0%
False negatives	0%	0%	0%
Positive predicted values	100%	100%	100%
Negative predicted values	100%	100%	100%
Accuracy (overall correct)	100%	100%	100%

The mean values and variance for the Stago® and Sysmex® analyses were 0.53 and 0.16, and 0.54 and 0.19, respectively. A Bland-Altman method comparison and a linear regression analysis (Figure 1) were also

performed to compare the results obtained on the same sample on the 2 analytical platforms before and after a single freeze-thaw cycle. The Bland-Altman analysis revealed an acceptable bias of at 0.008 IU/mL on the left axis and 0*z* on the right axis. Two observations lay outside the ±1.96 *z* either side. These 2 values were within the same critical value reference range (both were interpreted as therapeutic) and were discounted as they would not alter the clinical management. A correlation value of 0.99 (*P* = .001) was computed. The linear regression analysis revealed a model fit of 0.9846 and *P* < .001. A systematic deviation of 8% on the regression analysis was noted which was driven primarily by 4 values which lay outside the upper limit of the therapeutic reference range. As these tests were in agreement as being above the therapeutic reference range, they could be safely omitted from the analysis which altered the slope of the regression from 0.9192 to 0.9612, decreasing the underestimation from 0.0808 to 0.0388 and improving the regression model fit from 0.9846 to 0.9847. The limits of agreement in the Bland-Altman analysis also thus narrowed significantly from (−0.10 IU/mL; 0.13 IU/mL) to (−0.095 IU/mL; 0.09).

The Linear regression (a) and Bland-Altman plot (b) for initial,STA R Max®, and post-thaw, Sysmex CS-2500i®, anti-FXa analyses (Figure 1) were deemed acceptable with sufficient comparative stability between the two analysers to proceed with a secondary analysis of critical value ranges (Table 1). Based on the critical value interpretation, samples analysed pre- and post-thawing yielded identical results with a sensitivity, specificity and positive and negative predictive value of 100%.

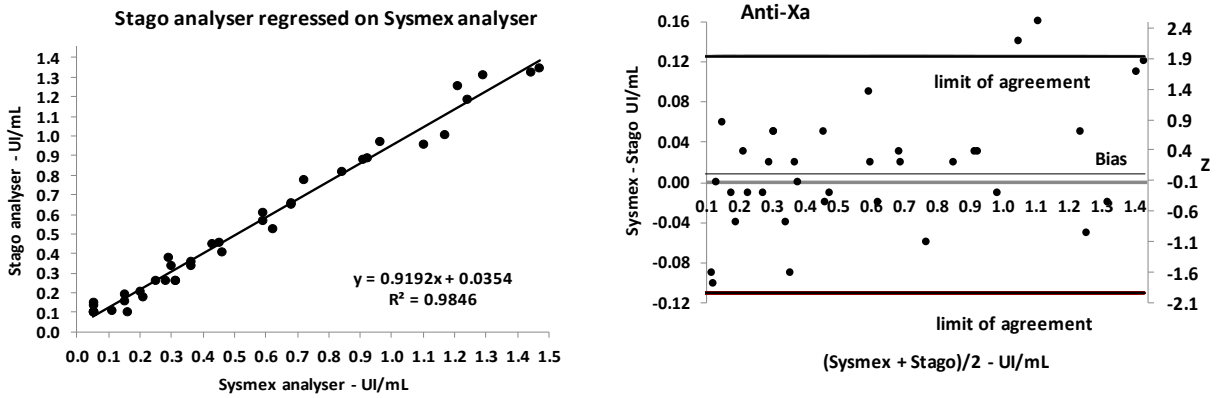

Figure 1: Linear regression (a) and Bland-Altman plot (b) and Sysmex vs. Stago

Anti-FXa analysis is an important monitoring tool in subsets of patients who are receiving LMWH anticoagulants, but this testing is not offered at smaller facilities and samples need to be frozen and transported to tertiary laboratories. This carries a risk of sample degradation. Coagulation assays are particularly sensitive to pre-analytical influences. To our knowledge, this is the first study that evaluates the effect of freezing on anti-FXa analysis utilizing two different analytical platforms, which are utilized within the NHLS laboratory network. Of note, the sample transport to facilities, which were distant from the central testing site, replicated the NHLS referral network. The testing showed a high correlation pre- and post freezing and transportation, and importantly, although results were not identical, the clinical interpretation remained consistent. This provides assurance that the laboratory practice of freezing plasma for anti-FXa analysis is acceptable and that results produced are accurate and comparable.

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