Usefulness of Cumulative Summation of Differences Method for Determining APTT Reagent Suitability

SUSAN H. FINDLATER

OBJECTIVE: The Cumulative Summation of Differences (CUSUM) is a recommended method for determining the consistency of one lot of Activated Partial Thromboplastin Time (APTT) reagent to another. This study investigates the usefulness of the CUSUM as a primary method for determining reagent suitability for APTT testing.

METHOD: Results for lot comparison, reference range and Ex-Vivo heparin sensitivity studies were obtained using the Beckman Coulter ACL TOP™ coagulation analyzer. APTT testing was performed using HemosIL™ SynthASiL w/CaCl and Heparin Xa testing was performed using the HemosIL™ Liquid Heparin Assay. Samples from normal patients and from patients taking heparin were tested.

RESULTS: The CUSUM calculation showed a difference in APTT reagent lot means that is within the acceptable range for this method, suggesting that the reagents were comparable. Reference range and heparin sensitivity studies demonstrated a clinically significant difference between the two reagent lot numbers tested.

CONCLUSION: The CUSUM method of evaluating reagent lot variation of APTT reagents should be used with caution as it may not completely reflect the performance of the reagent. Clinically significant differences between reagent sensitivity may not be detected. The results of reference range and heparin sensitivity studies should also be considered when determining the suitability of APTT reagents. In addition, due to research evidence that using the APTT test for monitoring patient anticoagulation therapy is problematic, an evaluation of the benefits of using other study methods and multiple study methods is suggested as well as continued examination of the use of the APTT as the test of choice for UF heparin monitoring.

ABBREVIATIONS: CUSUM - Cumulative Summation of Differences Method, APTT - Activated Partial Thromboplastin Time, UF - Unfractionated heparin, CAP - The College of American Pathologists, LIS - Laboratory information system, VRI - Verification of the reference interval, CLSI - Clinical and Laboratory Standards Institute

INDEX TERMS: Cumulative summation of differences, Activated Partial Thromboplastin Time, Reagent suitability, Heparin sensitivity

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***Susan H. Findlater , MHS, MLT, ART**, Clinical Chemistry, Department of Laboratory Medicine, Saint John Regional Hospital, Saint John, NB, Canada*

***Address for Correspondence: Susan H. Findlater, MHS, MLT, ART**, Clinical Chemistry, Department of Laboratory Medicine, Saint John Regional Hospital, 400 University Ave., Box 2100, Saint John, NB, Canada. E2L 4L2, Susan.Findlater@HorizonNB.ca*

INTRODUCTION

The Activated Partial Thromboplastin Time (APTT) is used to monitor heparin dosage amounts in patients receiving unfractionated (UF) heparin. It is recommended that reagents and instrumentation be adequately responsive to UF heparin if the APTT is being used for monitoring treatment.[1] UF heparin dosages are based on the range produced by performing an Ex-Vivo heparin sensitivity study. This range is calculated using a regression analysis that compares patients APTT results to Heparin Xa levels in order to determine the responsiveness of the APTT reagent to heparin. Using this Heparin Xa correlation method the APTT values that correspond to heparin Xa levels of 0.30 and 0.70 U/ml translate to the target range for therapeutic heparin levels. The Ex-Vivo method requires the collection of samples from patients that are currently receiving UF heparin and that fit specified criterion. Collection and testing of samples can prove

difficult for smaller laboratories due to difficulty in obtaining the appropriate number of test subjects and the limited availability of the Heparin Xa test.[1-4]

The College of American Pathologists (CAP) recommends that when a laboratory changes an APTT test method, the laboratory must determine the responsiveness of the method to UF heparin. According to the CAP Coagulation Resource Committee there is significant variability in responsiveness of APTT reagent to heparin due to reagent and patient differences.[1] Special attention should be paid to the heparin sensitivity of an APTT reagent in order to prevent issues where patients are over or under coagulated. The safe and effective use of heparin and attention to dosage administration is necessary in order to maintain the delicate balance between minimizing the risk of bleeding and the prevention of thrombosis formation.[5] A validation of the new reagent should be made by comparing it to the previous one in order to ensure that the reagent will produce APTT results in a similar range. Validations should also be done if there is a change of lot number of APTT reagent, change of heparin lot number used in the hospital, or a change in instrumentation.[6]

Comparison of heparin sensitivity of an APTT reagent with an existing and previously validated APTT reagent can be done using a cumulative sum of the differences between the result and a benchmark value (CUSUM). A mean difference or a cumulative change of more than seven seconds requires action and is reason for concern.

Actions include:

- Evaluation of another APTT reagent in an effort to find one that has a more acceptable variation level.
- Change the current therapeutic reference range to represent the difference in heparin sensitivity of the reagent.
- Perform additional heparin sensitivity testing.[3]

In May 2009, during the implementation of the ACL TOP instruments at the Saint John Regional Hospital an Ex-Vivo heparin sensitivity study and reference range determination was performed. In October 2010, a change in lot of SynthASiL was necessary due to the expiration of the current reagent. The CUSUM study, Ex-Vivo heparin sensitivity study and verification of the reference interval (VRI) were used for this initial reagent lot change with the consideration to move to using the CUSUM study as a primary method to investigate the acceptability of future lot changes.

MATERIALS AND METHODS

The results used for CUSUM study, Ex-Vivo heparin sensitivity study and VRI were obtained using the Beckman Coulter ACL TOP™ coagulation analyzer following the manufacturer's recommendations. APTT testing was performed using HemosIL™ SynthASiL w/CaCl and the Heparin Xa testing was performed using the HemosIL™ Liquid Heparin Assay. Samples were obtained from normal patients and from patients taking heparin. Samples were collected in 1.8 mL and 2.7 mL 0.109M BD Vacutainer Plus™, plastic 3.2% buffered Na Citrate blood collection tubes according to CLSI standards for collection, transport and processing of blood specimens using every effort to prevent preanalytical variables.[7,8]

Results were obtained by testing fresh plasma samples and double spun plasma aliquots frozen at -80°C. The EP Evaluator® statistics program, version 9.0, was used to analyze the study results. In this document, the current in use reagent will be referred to as APTT reagent A, the first APTT reagent lot to be studied will be referred to as APTT reagent B and the second APTT reagent lot to be studied will be referred to as APTT reagent C. The Ex-Vivo heparin sensitivity study was performed as follows on APTT reagent B, using a total of eighty-one plasma samples. Five samples were obtained from normal patients and seventy-six samples were drawn from heparinized patients collected according to specific criteria. Refer to Table 1 for sample selection criteria. APTT testing was performed on the original fresh samples and Heparin Xa testing was performed on the frozen samples. As a quality check for sample handling, APTT testing was also performed on the frozen samples and if the APTT results from the original and frozen samples differed by >10% the sample was discarded. The heparin therapeutic reference range was calculated using a regression analysis comparing the original APTT values for each sample to Heparin Xa levels to determine the responsiveness the APTT reagent to heparin. The APTT values that correspond to heparin Xa levels of 0.30 and 0.70 U/ml translate to the target range for therapeutic heparin levels.

Table 1. Sample selection for Heparin Therapeutic Range Study [1,9]

- 4-5 samples from normal patients (no anticoagulant).
- >30 samples from heparinized patients.
- Patients on the same type of heparin.
- No additional anticoagulants (Ex. Warfarin, Low Molecular Weight Heparin).
- Normal coagulation results before heparin (excludes patients with Lupus/Factor Deficiency).
- APTT results do not indicate obvious overheparinization.
- Use patient no more than twice.
- Plasma must have a platelet concentration of $<10 \times 10^9$/L.

A VRI study was performed on APTT reagent B, using plasma samples from twenty normal patients. Additionally eighty-eight normal patient samples were tested in an effort to increase the confidence of the data. The normal samples were a combination of fifty-seven fresh samples drawn from normal patients and fifty-one Precision Biologics Cryocheck™ Normal Donor frozen samples. An equal number of male and female samples tested. A CUSUM analysis was performed comparing APTT reagent A to APTT reagent B. The twenty-six plasma samples used for this APTT test comparison were collected using the same criteria as the samples collected from heparinized patients for the heparin sensitivity study. Refer to Table 1 for sample selection criteria.

A VRI study was performed as follows on APTT reagent C, using fifty plasma samples from normal patients. APTT testing was performed on a combination of fresh samples drawn from normal patients and Precision Biologics Cryocheck™ Normal Donor frozen samples. Samples were drawn from twenty three male and twenty seven female patients. A CUSUM analysis was performed comparing APTT reagent A to APTT reagent C. The twenty fresh plasma samples used for this APTT test comparison were collected using identical criteria as the samples collected from heparinized patients for the heparin sensitivity study. Refer to Table 1 for sample selection criteria.

RESULTS

The Saint John Regional Hospital implemented the Beckman Coulter ACL TOP™ coagulation analyzer in May 2009; the results being discussed here represent the first APTT reagent lot number change following this implementation. The Ex-Vivo heparin sensitivity study was performed on APTT reagent B using a total of eighty-one samples. The heparin therapeutic range was calculated using regression analysis comparing original APTT results to Heparin Xa levels in order to determine the responsiveness the APTT reagent to heparin. The APTT results used for this study ranged from 24.0-92.2 sec. and heparin Xa results ranged from 0.00-1.02 U/ml. The coefficient of determination (R^2) was 0.7712. Heparin levels of 0.30 and 0.70 U/mL. corresponded to APTT results of 48 and 92 sec., respectfully. (See Figure 1) In comparison to the results obtained for APTT reagent A upon implementation. The APTT results ranged from 25.7-97.2 sec. and heparin Xa results ranged from 0.00-0.77 U/ml. The coefficient of determination (R^2) was 0.7685. Heparin levels of 0.30 and 0.70 U/mL. corresponded to APTT results of 56 and 99 sec., respectfully. (See Figure 2)

The VRI study performed on APTT reagent B, using plasma samples from twenty normal patients failed the verification test with 10.2 % of the APTT results falling outside of the established reference interval. Adding an additional eighty-eight samples, while improving the confidence of the data, did not result in a successful VRI study. There is a demonstrated shift in sensitivity at both ends of the range, lack of verification of the reference interval and a distinct left shift evident in the statistical histogram. (See Figure 3) A comparison of APTT reagent lot A to APTT reagent lot B was performed using plasma samples drawn from twenty six patients receiving UF heparin. The resulting bias of -4.5 sec. confirmed that APTT reagent B was less sensitive to heparin than APTT reagent A. Further testing of APTT reagent B was suspended as a result of these preliminary results and APTT reagent C was obtained from the Vendor for analysis.

The VRI study was performed on APTT reagent C, using plasma samples obtained from fifty normal patients. This study passed the verification test with 4.0 % of the APTT results falling outside of the established reference interval. The reference interval result of 24.1-35.3 sec. was obtained and was considered to be statistically equivocal to the currently established range of 25.1-37.6 sec. A slight left shift in the histogram was not considered clinically significant based on the passing results of the VRI study. (See Figure 4) A comparison of result tested using APTT reagent lot A to APTT reagent lot C was performed using plasma samples drawn from twenty patients receiving UF heparin. The resulting bias

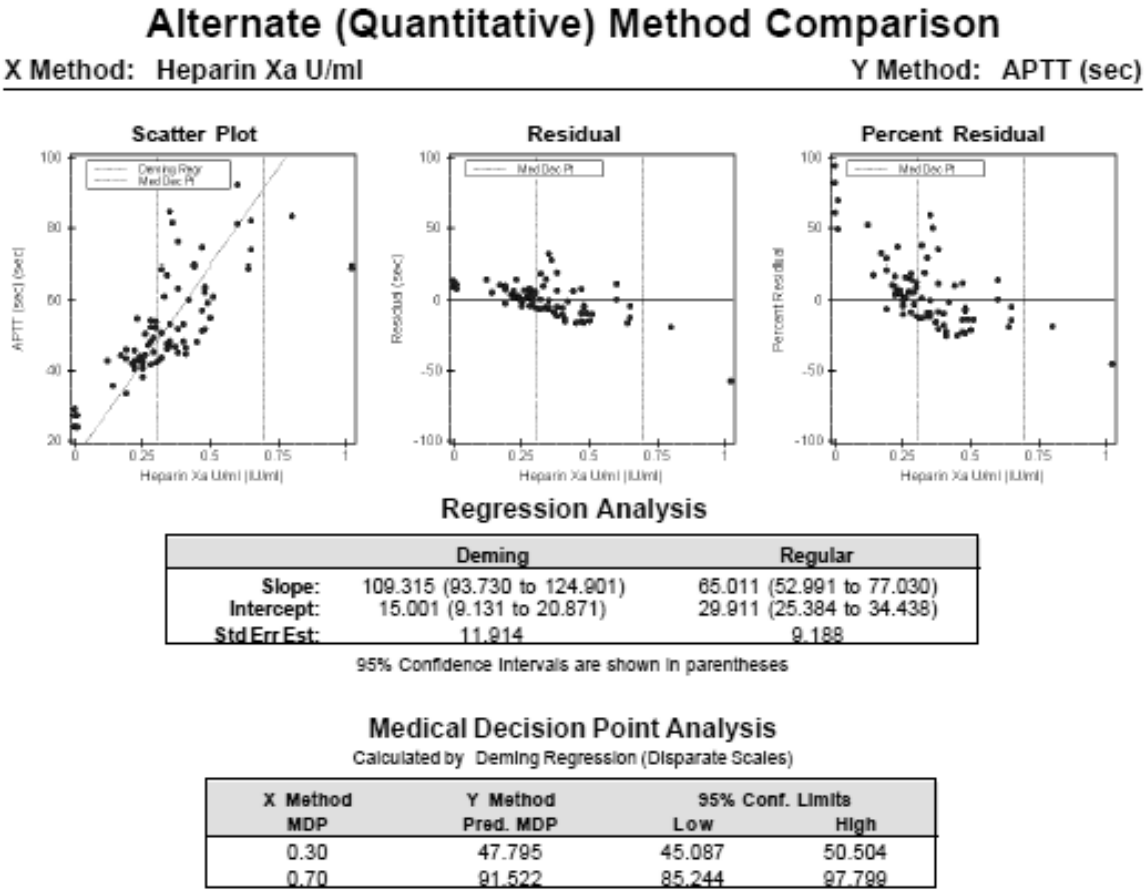

Figure 1. Heparin Sensitivity Study Reagent B

of -0.6 sec. confirmed that APTT reagent C has a similar sensitivity as APTT reagent A. Further studies were not performed at this time.

DISCUSSION

The CUSUM method is one of three CAP recommended methods for determining responsiveness of a reagent and/or instrumentation to UF heparin. Historically, our laboratory performed heparin sensitivity and VRI studies in order to determine reagent lot comparability. For the first reagent lot change following implementation of the Beckman Coulter ACL TOP™ coagulation analyzer, we considered changing our processes and performing the CUSUM method as a standalone method for the determination of our reagent suitability. The decision was made to perform the heparin sensitivity and VRI as a validation of the CUSUM method before initiating this change.

APTT reagent lot B was the first lot to be compared to the current reagent being used, APTT reagent lot A. The heparin sensitivity study performed using APTT reagent B resulted in the determination of a heparin therapeutic range of 48-92 sec. which is statistically different from the established heparin therapeutic range of 56-99 sec. obtained on the current reagent A. If APTT reagent B was in use an adjustment to the heparin therapeutic range would be necessary. The lower end of the heparin therapeutic range of APTT reagent B at 48 sec. created concern due to the possibility of confusion and the close proximity to the current APTT reagent reference intervals upper range of 37.6 sec.

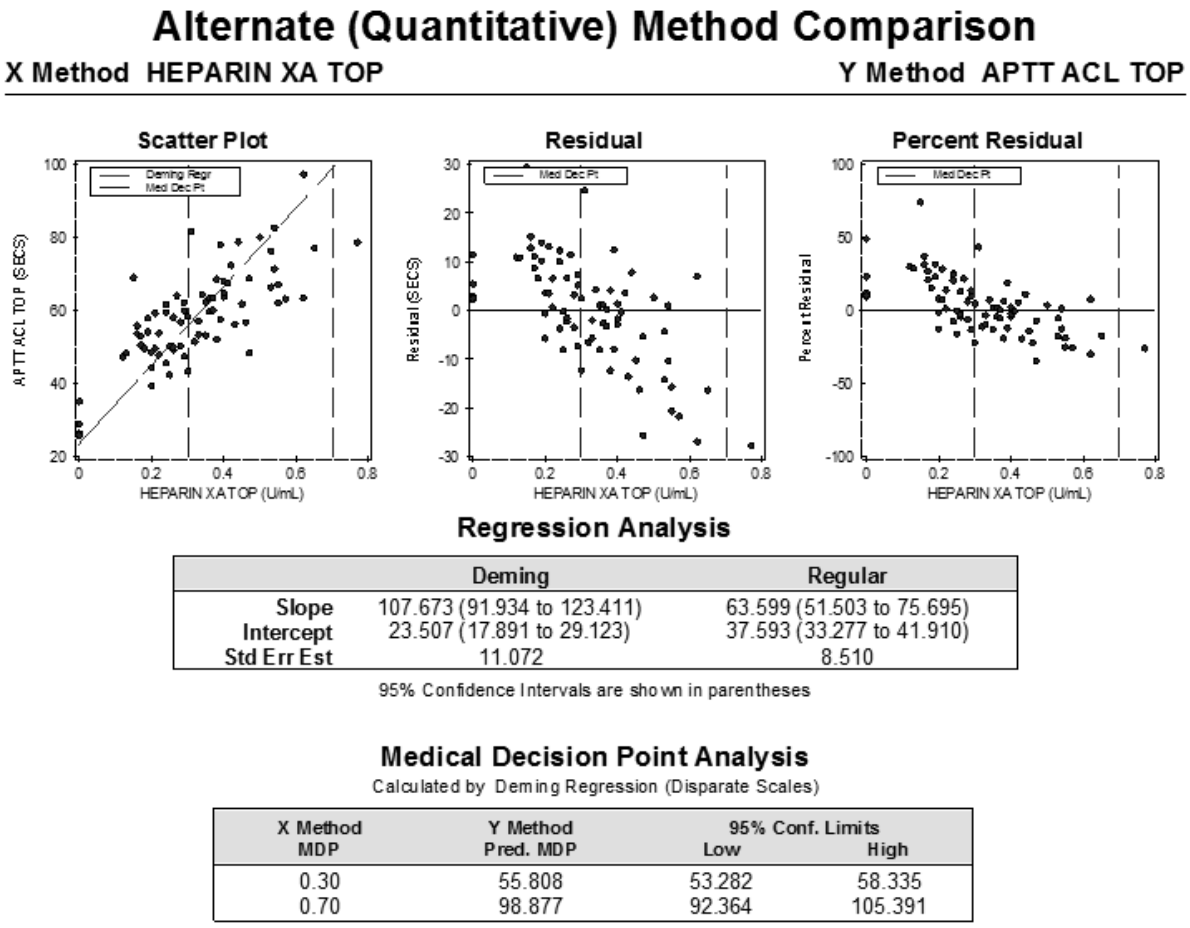

Figure 2. Heparin Sensitivity Study Reagent A

The outcome of the VRI on APTT reagent B resulted in similar results when compared to the established reference interval for APTT reagent A. Changing the reference interval from 25.1-37.6 sec to 23.1-31.9 sec. would be an adjustment for Clinicians and has the potential to create problems for the monitoring of patients treatments. In addition to these changes, the laboratory established APTT level used for the investigation of circulating anticoagulants which uses the method of adding 5 seconds to the APTT range upper value would be adjusted to 36.8 sec. from the currently used value of 43.8 sec.[1]

The lot to lot comparison of APTT reagent B to APTT reagent A resulted in a bias of -4.5 seconds, which is within the specifications for the CUSUM method for the verification of lot suitability. Additional issues that surround the shift in mean reference range values are lowered sensitivity to heparin and the potential to affect patient treatment and care. The VRI for reagent B failed verification; additional samples were tested in an effort to increase the confidence of the data. The total number of normal samples tested was one hundred and eight which is twelve less than the one hundred and twenty samples recommended by CLSI standards for the determination of Reference Ranges. This could be considered a limitation to this study.[9,10]

Changes to laboratory reports, laboratory information system (LIS) adjustments and communication to clinicians educating them about the change and resulting relevance to patient treatment would be necessary if the decision was made to accept APTT reagent B. It was concluded that implementation of APTT reagent B had the potential for increased risk of over-heparinization of patients in addition to the possibility of treatment difficulties due to the long history and familiarization of Clinicians to the current reference intervals and the current heparin therapeutic range.

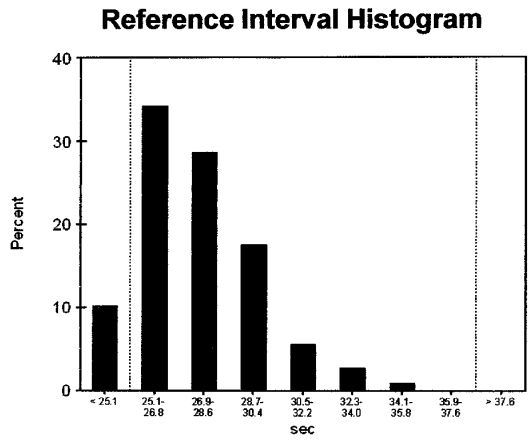

Figure 3. VRI Study Reagent B

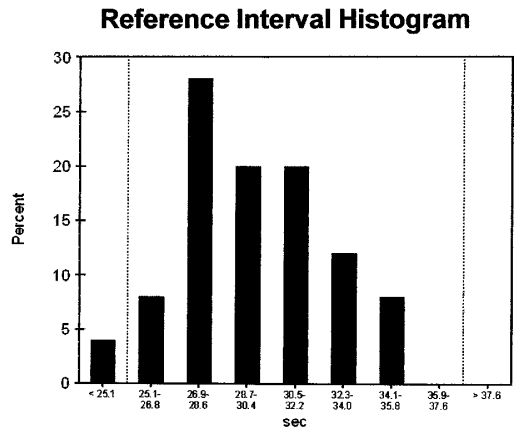

Figure 4. VRI Study Reagent C

The studies performed on APTT reagent C resulted in a successful VRI study. This meant that if APTT reagent C was implemented a change in Reference Interval for APTT would not be necessary. When APTT reagent C was compared to APTT reagent A using the CUSUM method of lot to lot comparison, the result was a bias of -0.6 sec. APTT reagent C proved to be similar in sensitivity to the current APTT reagent A. APTT reagent C proved to be suitable for application in our laboratory and by doing so we were able to avoid unnecessary changes in current protocol and lessened the risk of treatment errors for patients.

Research evidence has shown that monitoring the effectiveness of UF heparin with the APTT test is problematic. A study by Raschke, Hirsh & Guldry identified the association of suboptimal dosing and monitoring of UF heparin to effectiveness in treating patients with venous thrombosis, but evidence could not be linked by the data obtained in the study to a worsened clinical outcome by patients.[11] Noticeable effects of preanalytical variables on APTT test results were identified in a study by McGlasson et al. Also determined by this study, when the APTT test is used for heparin management, inappropriate treatment may occur creating the potential for life threatening complications.[12] Due to variances in APTT reagents it has also been identified in a study performed by Eiklboom & Hirsh that laboratories may have difficulty providing an accurate test range used for treatment and monitoring of heparin therapy, creating difficulty for clinicians to treat patients effectively.[13]

In order to determine if the acceptability range of 7 sec. is indeed too broad for determining the suitability of reagents, additional research would be useful surrounding the application of the CUSUM method of determining APTT reagent lot suitability. Clinically significant differences between reagent sensitivity may not be detected when the recommended application of a reagent sensitivity difference of 7 sec. is used. The results of VRI studies and heparin sensitivity studies using the Ex-Vivo method should also be considered when determining the APTT reagent suitability. The CUSUM method of evaluating reagent lot variation of APTT reagents should be used with caution as it may not completely reflect the performance of the reagent. In addition, due to evidence that using the APTT test for monitoring patient anticoagulation therapy is problematic, an evaluation of the benefits of using other study methods and multiple study methods is suggested as well as continued examination of the use of the APTT as the test of choice for UF heparin monitoring. Efforts to streamline the investigation of reagent suitability while keeping with current recommended quality standards would be useful to laboratories.

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