# Are the Point-of-Care Diagnostics MULTIPLATE and ROTEM Valid in the Setting of High Concentrations of Heparin and Its Reversal With Protamine?

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***Objectives:*** **To evaluate the in vitro effects of high concentrations of heparin and its reversal with protamine on routine laboratory parameters as well as on modified thromboelastogram (ROTEM; TEM International, Munich, Germany) and impedance aggregometry (MULTIPLATE; Dynabyte, Munich, Germany).**

***Design:*** **An observational, nonrandomized in vitro study.**

***Setting:*** **A single-center, university hospital.**

***Participants:*** **Ten healthy volunteers.**

***Interventions:*** **Heparinization of whole blood to levels of 2, 4, 6, and 8 IU/mL of heparin and reversal with protamine. For MULTIPLATE measurements, heparin levels up to 20 IU/mL were tested.**

***Measurements and Main Results:*** **The present results show that the prothrombin time (PT) and fibrinogen measurements are altered significantly by heparin concentrations above 2 IU/mL. Protamine reversal also affected coagulation tests except for the fibrinogen. The INTEM test using the ROTEM system was influenced significantly by heparin concentrations of 2 IU/mL or higher, whereas EXTEM measurements remained stable up to 4 IU/mL. The findings for the FIBTEM test were stable up to 6 IU/mL but then declined to values less than 50% of baseline at 8 IU/mL. HEPTEM results remained valid under all concentrations of heparin tested. The effect of protamine on ROTEM was seen mainly in the INTEM and HEPTEM measurements. Heparin concentrations up to a level of 20 U/mL had no effect on MULTIPLATE measurements. Effects of protamine on MULTIPLATE became significant at heparin-to-protamine ratios below 1:1 and were more pronounced for adenosine diphosphate than for thrombin receptor-activated protein testing.**

***Conclusions:*** **Neither fibrinogen (Clauss) nor derived fibrinogen or FIBTEM testing is valid in the setting of high concentrations of heparin unless antagonized by heparinase. Reversal of heparin with protamine worsens platelet function at all ratios as detected by aggregometry (MULTIPLATE) and thromboelastography (ROTEM), starting at a 1:1 ratio. Therefore, appropriate coagulation testing under cardiopulmonary bypass conditions should be selected carefully according to heparin levels. In particular, fibrinogen values are falsely low at heparin levels of 2 IU/mL and above. Therefore, newer algorithms promoting the correction of fibrinogen levels on cardiopulmonary bypass should be based on appropriate testing.**

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TRADITIONALLY, most coagulation tests such as platelet count, prothrombin time (PT), activated partial thromboplastin time (PTT), and fibrinogen levels are performed routinely on citrated plasma samples in the central laboratory. Newer techniques to improve and accelerate coagulation monitoring at the bedside using whole blood have been established and are gaining widespread acceptance, particularly for cardiac surgery patients. Thromboelastography has long been considered a valuable predictor of postoperative blood loss[1] and as a guide to hemostatic management after bypass.[2] This has been the authors' practice for the last 2 years.

Recent reports propose the use of the ROTEM system (TEM International, Munich, Germany) to correct acquired coagulation deficiencies even while the patient is still on bypass with high heparin concentrations.[3,4] However, the authors have observed a significant variability over time in modified thromboelastometry (ROTEM) and platelet aggregometry (MULTIPLATE; Dynabyte, Munich, Germany) during cardiopulmonary bypass.

The authors hypothesized that high heparin concentrations, as high as 7 IU/mL, may be the cause of the observed variability. Average heparin levels on cardiopulmonary bypass usually run between 1.3 and 7.2 IU/mL in adults.[5] Therefore, the authors designed a study to determine the accuracy and validity of ROTEM and MULTIPLATE monitoring in the setting of high heparin concentrations and its reversal with protamine. Additionally, the authors looked at the effect of heparin and protamine reversal on routine coagulation monitoring. An in vitro titration model was generated to cover most clinical scenarios of heparin and protamine concentrations during on-pump cardiac surgery.

## METHODS

Ten healthy volunteers were recruited from the authors' department, and informed consent was obtained. All refrained from taking any medication that could possibly interfere with coagulation or platelet function for more than a week. Institutional review board approval was waived because of the in vitro nature of the study.

Blood was drawn from a venipuncture site with a 21-G butterfly needle into 3.8-mL tubes containing 0.38 mL (0.129 mol/L) buffered (pH 5.5) sodium citrate (Sarstedt, Nuembrecht, Germany) for ROTEM and standard coagulation testing and 2.7-mL tubes containing 1.6 mg of EDTA per milliliter of blood (Sarstedt, Nuembrecht, Germany) for complete blood counting. For the MULTIPLATE measurements, blood was drawn into hirudin-prefilled tubes (25 $\mu$g/mL) and rested for 30 minutes before analysis as recommended by the manufacturer. ROTEM tests were run immediately.

The Institute of Laboratory Medicine processed the standard laboratory tests. Activated PTT (Pathromtin SL), PT (Innovin), and fibrinogen concentration (modification of the Clauss method using Multifibren U) were determined on the BCS analyzer (instrument and reagents from Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Blood cell counts were measured using the whole-blood counter XE 2100 (Sysmex, Kobe, Japan). PTT values greater than 150 seconds were treated as no clotting. PT-derived fibrinogen estimation was

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performed using the manufacturer's calibration method. The derived fibrinogen technique uses the difference in light scattering before and after clot formation compared with the readings taken using a known fibrinogen concentration and extrapolates the fibrinogen concentration. Therefore, PT-derived fibrinogen is not a direct measurement of fibrinogen.

To examine the effect of heparin and protamine on coagulation and ROTEM tests, heparin (Heparin-Natrium 10,000 IU/mL; B Braun, Melsungen, Germany) was added to aliquots of citrated blood samples to obtain final serum heparin concentrations of 2 IU/mL, 4 IU/mL, 6 IU/mL, and 8 IU/mL.

Using samples containing a concentration of 4 IU/mL of heparin, antagonism with protamine at 1:1, 1:1.5, and 1:2 ratios was established to test the real-life effect of daily clinical practice. The starting heparin concentration was chosen based on average heparin levels on adult cardiopulmonary bypass.[5] To investigate the effects of heparin and protamine on MULTIPLATE analysis using anticoagulated whole blood, heparin concentrations of 5 IU/mL, 10 IU/mL, and 20 IU/mL were chosen; the authors antagonized the 10-IU/mL aliquot with protamine in a 1:1, 1:1.5, and 1:2 ratio. In a similar fashion, the protamine effects on all coagulation tests were determined by adding 20 $\mu$L of protamine to the sample tubing. Dilutional effects were kept constant throughout and were less than 1%.

ROTEM measurements were performed according to the manufacturer's recommendations. Technical details and a comparison with the TEG system (Haemoscope Corp, Niles, IL), are presented elsewhere.[6,7] In brief, clot formation is measured at 37°C after recalcification of 300 $\mu$L of whole blood (20 $\mu$L of calcium chloride 0.2 mol/L) and the addition of different reagents to shorten the reaction time and to differentiate between hemostatic disorders including contact activation with thromboplastin-phospholipid (20 $\mu$L) in the INTEM test, specific elimination of heparin by adding 10 $\mu$L of heparinase type I to the above for the HEPTEM test, tissue factor activation by adding 20 $\mu$L of thromboplastin reagent prepared from rabbit brain for monitoring the extrinsic system (EXTEM), and lastly the combination of tissue factor activation with platelet inhibition using cytochalasin D for qualitative analysis of fibrin clot stability (FIBTEM). ROTEM parameters shown in the figures and tables were the coagulation time (CT) and maximal clot firmness (MCF) (Fig 1 and Table 2 and 3).

To assess platelet function, the commercial whole-blood platelet function analyzer MULTIPLATE was used. The technique is based on the principle of impedance aggregometry and measures resistance changes between 2 electrodes immersed in whole blood. Platelet aggregates around the electrodes increase the impedance between them. The change of the impedance is transformed to arbitrary aggregation units and plotted against time. The system uses activation by adenosine diphosphate and thrombin receptor-activating protein (TRAP) for the assessment of platelet function in whole-blood samples; 300 $\mu$L of blood is mixed with 300 $\mu$L of prewarmed isotonic saline solution. After incubation for 3 minutes, 20 $\mu$L of activating substrate are added to the sample. Activated platelet function is recorded for 6 minutes. Duplicate measurements of each sample are run simultaneously. The computer analyzes the area under the curve (AUC) of each sample and calculates the mean values. The authors performed testing using either ADP (ADP test 0.2 mmol; Instrumentation Laboratory, Munich, Germany) or TRAP activation agents (TRAP test 1 mmol, Instrumentation Laboratory).

As shown previously, $n \geq 6$ is a sufficient number to detect differences in thromboelastometric variables with a power $\geq 0.8$ and a significance of $\leq 0.05$.[8] Data are given as mean $\pm$ standard deviation. After testing for normal distribution (Kolmogorov-Smirnov test), a paired Student $t$ test was used to compare changes against baseline (100%) and against previous concentration points. A $p$ value $<0.05$ was considered statistically significant. A Bonferroni correction as needed for multiple testing was performed but did not reveal any different results from crude testing. Therefore, raw $p$ values are reported. All statistics were performed using PASW Statistics 18.0 (SPSS Inc, Chicago, IL).

## RESULTS

Of the 10 test subjects, 2 were female. None of the test subjects suffered from subclinical cell counts or coagulation abnormalities that could interfere with testing.[9,10] Baseline values for complete blood counts were a hemoglobin of 14.0 $\pm$ 0.8 mg/dL and a platelet count of 218,000 $\pm$ 31,000/$\mu$L. Baseline coagulation values are presented in Table 1 and Figure 1.

PT, PTT, and fibrinogen Clauss and derived fibrinogen measurements were influenced significantly by heparin concentrations starting at 2 IU/mL (Fig 1). Protamine reversal also significantly affected PT, PTT, and derived fibrinogen measurements in a dose-dependent manner. Only the fibrinogen Clauss test remained stable throughout antagonism (Table 1).

On INTEM testing as well as during PTT testing, no coagulation was observed at heparin concentrations of 2 IU/mL and higher (Fig 1 and Table 2). EXTEM measurements were stable up to 2 IU/mL of heparin at which point CT and clot formation time were prolonged significantly, but MCF remained stable up to heparin concentrations of 4 IU/mL. The findings for the FIBTEM test were similar, with no effect until more than 4 IU/mL of heparin. However, 6 IU/mL and 8 IU/mL of heparin significantly reduced Fibtem values to 72% and 45% of baseline, respectively (Table 2).

The effects of protamine on ROTEM mainly show an influence on INTEM and HEPTEM measurements and to a lower

**Table 1. Effect of Protamine on Laboratory Values**

| Group | PT (s) | PTT (s) | Fibrinogen Clauss (mg/dL) | Derived Fibrinogen (mg/dL) |
|---|---|---|---|---|
| Baseline | 8.1 $\pm$ 0.3 | 33 $\pm$ 4 | 316 $\pm$ 57 | 281 $\pm$ 51 |
| H:P 1:1 | 10.8 $\pm$ 1.4† | 57 $\pm$ 17 | 313 $\pm$ 51 | 303 $\pm$ 57† |
| H:P 1:1.5 | 12.8 $\pm$ 1.7*† | 77 $\pm$ 30† | 323 $\pm$ 48 | 356 $\pm$ 63† |
| H:P 1:2 | 14.8 $\pm$ 0.7*† | 124 $\pm$ 29*† | 322 $\pm$ 53 | 387 $\pm$ 77† |
| Protamine, 20 $\mu$g/mL | 29.9 $\pm$ 3.8*† | 150 $\pm$ 0*† | 343 $\pm$ 53 | 609 $\pm$ 125*† |

NOTE. The effect of protamine on laboratory tests is shown after antagonism of 4 IU/mL of heparin with protamine in a 1:1 to 1:2 ratio (H:P 1:1, 1:1.5, 1:2) or protamine 20 $\mu$g/mL only. Values are shown as mean $\pm$ standard deviation. The upper limit of PTT is 150 seconds.

Abbreviation: NC, no clot.

*$p < 0.05$ versus previous value.

†$p < 0.05$ versus baseline value.

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