Heparin is administered as an anticoagulant in treatment of or prophylaxis against arterial or venous thromboembolism, in treatment of myocardial infarction, and during cardiac surgery (cardiopulmonary bypass) [1], coronary angioplasty, and other procedures [2].

Heparin treatment demands laboratory control and is monitored by determining the activated partial thromboplastin time (APTT), which should be adjusted to stay within 1.5–2 times the control value [3]. Sometimes during therapy high APTT values (>180 s) are reached [4], and physicians decide to interrupt the treatment for a few hours because of the great risk of hemorrhagic complications, the most important side effect of heparin therapy [5]. In these situations, however, the true concentrations of heparin in the patient’s plasma are really unknown. Moreover, in some described cases, the APTT values were prolonged because of underlying disease (severe liver disorders, extensive myocardial infarction, or infection) and postoperative complications after cardiac surgery [6]. This prolongation of APTT was not heparin related, although the results of the APTT test falsely suggested a higher heparin concentration [7]. Another problem is that sensitivity of the reagents for the APTT test varies greatly, and the physicians who prescribe heparin on the basis of the test are uncertain whether the heparin concentration is really high or low [8].

In cases with the patient’s APTT >180 s, the use of a plasma heparin assay may in fact be better for anticoagulation control. That is, measurement of anti-Xa activity of heparin in plasma may be more specific for monitoring therapy and be superior to APTT determination [8, 9]. In the present study, we investigated the correlation between heparin activity in plasma (anti-Xa activity) and the incidence of bleeding complications in patients with APTT >180 s.

The procedures used were in accordance with the Helsinki Declaration of 1975, as revised in 1983. All 47 patients in our study (ages 62 ± 8.9 years, range 43–77) were treated with a continuous intravenous infusion of heparin for management of thromboembolic disorders or as a prophylaxis against any (Heparin Leo; Leo Pharma- ceutical, Ballerup, Denmark; 2500–5000 IU as a bolus dose and 1000 IU/h after).

In detail, four patients had acute myocardial infarction, five had unstable angina, one had rheumatic mitral valve disease, one had pulmonary embolism, and one had atrial fibrillation. Twenty-seven patients had undergone percutaneous transluminal coronary angioplasty, four cardiac catheterization, three coronary bypass surgery, and one valvuloplasty. Four of the 47 patients had thrombocytopenia at the time of the study. No patient had malignant or hematological disease or stroke, and all had normal renal function. These patients were not receiving any drug, besides heparin, that might alter the results of the anti-Xa activity or the APTT assay.

The APTT values of the patients were >180 s when the plasma samples of this study were collected. Any major and minor bleedings that happened during that time of heparin therapy were recorded. Bleedings were defined as major that led to a blood transfusion, to a decrease in hemoglobin by >24 g/L, or to formation of surgically treated hematomas; all other bleedings were defined as minor.

The plasma samples were collected into sodium citrate as anticoagulant (10:1 by vol. final concentration) with use of the Vacutainer Tube system (Hemogard tubes no. 367714; Becton Dickinson) and were separated by centrifuga- tion (at ambient temperature, 10 min, ~1500g) within 10 min from collection. They were stored at −70°C until assayed.

The APTT assay (CIT Neothrombin; Behring) was performed in a semiautomated instrument (Behring Chromo- timer) with a chromogenic substrate. According to this method the patient’s plasma (50 μL) is incubated with optimal amounts of phospholipids and an activator (25 μL). The result is the activation of the factors of the intrinsic pathway. Coagulation is then triggered by the addition of calcium ions. Absorbance at 405 nm increases with the formation of activated thrombin, and the result is determined on the basis of the time taken for this absorbance to increase by 0.1. The reference interval, according to the manufacturer, is 28 to 40 s.

To determine anti-Xa activity of unfractionated heparin (expressed in IU/mL), we performed the Berichrom Hepar- in assay (Behring) in the same instrument. In the incubation phase of this method, antithrombin III inactivates factor Xa in a reaction catalyzed by heparin. Dextran sulfate releases whatever heparin has bound to interfering factors and thus makes it accessible to the assay. The quantity of factor Xa remaining after the incubation is determined via the increase in absorbance at 405 nm, with use of a chromogenic substrate in a kinetic test. This sensitive assay is not disturbed from the possible abnormal concentrations of fibrinogen or fibrin/fibrinogen degrada- tion products.

The assay was calibrated in the range 0–1 IU/mL (0–1000 IU/L) by comparison with known plasma concentra- tions of heparin (Heparin Leo 5000 IU/mL, i.e., 5 × 106 IU/L in Behring’s human plasma calibrator). Concentra- tions >1 IU/mL were determined after diluting the plasma with an equal volume of isotonic saline.

All 47 patients treated with heparin had APTT values >180 s. Their mean anti-Xa activity (±SD) was 0.94 ± 0.21 IU/mL, within a range of 0.54–1.29 IU/mL, and 8 patients had anti-Xa values within the therapeutic range of 0.35–0.7 IU/mL. In 18 patients (38.3%), the heparin treatment led to bleeding complications, 11 minor (23%) and 7 major (15%).

Patients were then categorized in two groups: the 26 whose anti-Xa activity was <1 IU/mL and the 21 with
anti-Xa activity >1 IU/mL (>1000 IU/L). The risk of bleeding in these two groups was found to be 23% and 57%, respectively (Table 1). The difference between incidences of bleeding (%) in the groups was statistically significant ($P = 0.037$, Yates corrected $\chi^2$ test). Major bleeding complications were seen in 3 of the 26 patients (11.5%) with anti-Xa activity <1 IU/mL and in 4 of the 21 patients (19.0%) with anti-Xa activity >1 IU/mL (not significantly different).

Our results indicate that the incidence of bleeding risk was significantly increased in patients with anti-Xa activity >1 IU/mL. Given that in patients with APTT >180 s the exact degree of anticoagulation is unknown, anti-Xa activity may be clinically informative and can contribute to the control of bleeding complications. Moreover, interruption of treatment in patients with APTT >180 s, who have therapeutic concentrations of plasma heparin, leads to inadequate protection against thromboembolic disease.

### Table 1. Frequency of bleeding in the two patient groups with anti-Xa activity < or >1 IU/mL.

<table>
<thead>
<tr>
<th>Anti-Xa activity, IU/mL</th>
<th>With bleeding</th>
<th>Without bleeding</th>
<th>Total</th>
<th>Risk, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>6</td>
<td>20</td>
<td>26</td>
<td>23.0</td>
</tr>
<tr>
<td>&gt;1</td>
<td>12</td>
<td>9</td>
<td>21</td>
<td>57.1</td>
</tr>
</tbody>
</table>

a These frequencies are statistically significantly different ($\chi^2$ test, $P < 0.037$)

CryoSeal™ System, a New Device for Generating Cryoprecipitate from Plasma, R.C. Gosselin,* E. Larkin, J.T. Otings, and P. Coehlo† (Univ. of California, Davis Med. Ctr., Sacramento, CA, and 1 Thermogenesis Corp., Rancho Cordova, CA; *address correspondence to this author at UC Davis Med. Ctr., 4625 2nd Ave., Rm. 3203, Sacramento, CA 95817: fax 916-734-3320, e-mail bgosselli@clb.ucdm.ucdavis.edu)

The CryoSeal™ System (CSS; Thermogenesis Corp.) is a device that can prepare freeze–thaw cryoprecipitate (Cryo) generated from plasma in ~1 h. Traditional blood bank preparations of Cryo contain high concentrations of anti-hemophilic factor (plasma coagulation factor VIII) and fibrinogen, but require several days, numerous pieces of laboratory equipment, and considerable staff handling to prepare [1]. We investigated whether CSS-prepared material is substantially equivalent in factor VIII and fibrinogen contents to the traditionally prepared Cryo material.

Whole blood drawn from volunteer donors ($n = 16$) at the Sacramento Medical Foundation Blood Center (SMFBC), with use of citrate–phosphate–dextrose–adenine anticoagulation, was centrifuged at 2500g for 5 min at 4 °C within 6 h of collection. The units of plasma were then used for preparation of Cryo in the CSS.

The CSS consists of a thermodynamic device featuring a temperature-controlled rocker plate and a clear, plastic, single-use plasma-processing container that rests on the rocker plate. Upon initiation of Cryo processing, the CSS automatically transfers the donor plasma from a transfer pack to the processing container by a peristaltic pump in a closed system of tubing. The CSS then lowers the temperature and sets the plate in motion to rapidly freeze the plasma to ~27 °C. The rocker plate temperature is then raised to 2 °C to thaw the plasma while concurrently rocking the plate in a manner that causes the insoluble clotting and adhesive proteins of Cryo to agglomerate and migrate to the tip of the processing container for removal by the attached syringe. The residual Cryo-poor plasma is then automatically transferred back to the transfer pack to yield a Cryo volume of ~10.0 mL. This Cryo sample was submitted for factor VIII activity and fibrinogen analysis.

Immunodepleted factor VIII-deficient plasma (Dade International) was used for all factor assays. CryoCheck (Precision Biologicals) was the factor VIII calibrator used for donor samples, and US Standard Anticoagulant Factor Mega 1 was used as the calibrator for Cryo. All tests were performed on the MLA 1000C coagulation analyzer (Medical Laboratory Automation) with Actin FS (Dade) aPTT reagent. Each factor calibrator was automatically prepared by the MLA 1000C by sequentially diluting the calibrator seven times with buffered saline (barbital buffer; Dade) to dilutions from 1:5 to 1:320. The subsequent test samples were diluted 3 times to give dilutions from 1:5 to 1:20. Each calibration dilution was tested in duplicate and the replicate results had to match within 5% to be accepted as a valid point. All factor results were reported in units/mL or total units (factor VIII units/mL multiplied by volume).

Fibrinogen was determined by the modified Clauss [2] method by using commercially prepared thrombin (Dade). College of American Pathologists Fibrinogen Reference plasma was used to calibrate the fibrinogen calibration curve. The fibrinogen calibrator was automatically prepared by the MLA 1000C by sequentially diluting the reference material 5 times with barbital buffer, creating dilutions from 1:3.5 to 1:40. The fibrinogen calibrator was analyzed only once before sample testing. Each fibrinogen calibrator and test plasma were analyzed in duplicate, with agreement within 5% required. Cryo samples were diluted 1:5 with buffered saline before analysis, and the