

**Platelet and Leukocyte Counts as Hemocompatibility Indicators for Biomaterials:  
An Evaluation of ASTM Standard Test Method F2888-13**

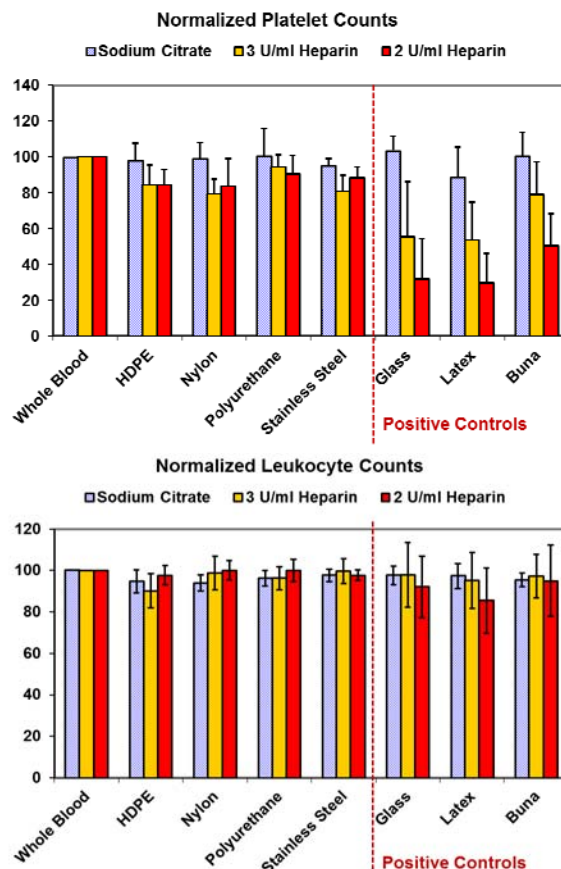
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**Statement of Purpose:** To reduce thrombosis related adverse events for patients exposed to blood-contacting medical devices, it is critical to fully evaluate thrombogenicity as part of the non-clinical hemocompatibility testing of medical materials. Recently, a standardized test method (*ASTM F2888-13: Standard Method for Platelet Leukocyte Count — An In-Vitro Measure for Hemocompatibility Assessment of Cardiovascular Materials*) was published to assist in the evaluation of cardiovascular device materials for their ability to induce thrombus formation. The goals of this project were to evaluate whether the standard method described in ASTM F2888 is capable of differentiating materials with different thrombogenic potentials, and to determine the effects of using different anticoagulants on the test results.

**Methods:** Testing with sodium citrate anticoagulation was performed according to ASTM F2888-13. Briefly, fresh human whole blood (obtained from the National Institutes of Health Blood Donor Research Program) anticoagulated with 3.2% sodium citrate was incubated with the test materials (in a ratio of 1mL of blood to 12 cm<sup>2</sup> of material) for 60 minutes at 37 °C in an agitating water bath. After the 1 h incubation period, EDTA was added to the blood at a final concentration of 5 mM to inhibit further reactions. Each blood sample was then transferred to a new tube and placed on ice until the platelet and leukocyte counts were measured using an automatic hematology analyzer (HV950FS from Drew Scientific). The tested materials included some commonly used biomaterials (Polyurethane, Nylon, HDPE, 316L Stainless Steel) and positive controls (Glass, Buna-N Rubber, Latex). The negative control was whole blood subjected to the above treatment but without contact with the test materials. To investigate the effects of different anticoagulation conditions, testing was also carried out with the same procedures as described above with the following modifications on blood anticoagulation: Immediately before adding blood to the test materials, sodium citrated blood was recalcified with calcium chloride (final concentration of 10 mM), then heparin was added to the blood to obtain a final concentration of 2 U/ml and 3 U/ml. Blood from 6 donors was used, and each donor's blood was tested in duplicate against each test material for all anticoagulation conditions.

**Results and Discussion:** Platelet and leukocyte count results (normalized to their corresponding whole blood negative controls) with different anticoagulation conditions are shown in Figure 1. For sodium citrate anticoagulant, platelet count results for all materials were unchanged and roughly 100% of the whole blood control.



**Figure 1** Platelet counts (top) and leukocyte counts (bottom) normalized to whole blood control. N=6 donors.

In contrast, the heparin anticoagulation allowed for differentiation between positive controls and commonly used biomaterials when assessed using the platelet count. Compared to 3 U/mL heparin, 2 U/mL heparin showed a slightly better differentiation between the positive controls and biomaterials. However, leukocyte counts were approximately the same for all materials and did not distinguish between the positive controls and the test materials, regardless of the anticoagulation conditions.

**Conclusions:** Our data indicate that the use of sodium citrate anticoagulation as stipulated in the ASTM F2888 standard is not effective in differentiating materials with different thrombogenic potentials. However, the modification to use low concentration heparin allowed us to discern between the positive controls and commonly used biomaterials when using platelet count as the indicator. Leukocyte count did not show any difference between the test materials for all tested anticoagulants and thus is not a sensitive indicator to evaluate thrombogenic properties of biomaterials.