User Manual: Performing Platelet and Leukocyte Count Assays for In Vitro Hemocompatibility Assessment of Cardiovascular Materials per ASTM F2888-19 Standard

Tool Reference

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Performing Platelet and Leukocyte Count Assays for In Vitro Hemocompatibility Assessment of Cardiovascular Materials per ASTM F2888-19 Standard

This document provides details that will be helpful in successfully performing thrombogenicity screening of biomaterials and medical devices per ASTM F2888-19. This document augments ASTM F2888-19 and contains the following details:

i) Test methodology for performing platelet and leukocyte count assays
ii) Details about choice of positive controls
iii) Adjustment of blood anticoagulant concentration
iv) Data for platelet and leukocyte assays for different anticoagulation strategies
v) Discussion on test sensitivity for platelet and leukocyte count assays

i) Test Methodology

The "MATERIALS and METHODS" section of Lu et al. 2021 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8490275/) contains all the details about experimental setup, test components, anticoagulation strategy, and test procedures for both platelet and leukocyte count assays

Test Protocol

For detailed step-by-step test protocol, please follow Sections 9,10, and 12 of ASTM F2888-19 standard. Additional details to supplement the test method are provided here.

Human blood acquisition

Venous blood from healthy donors was drawn into polypropylene tubes containing either 3.2% sodium citrate (blood to sodium citrate solution volume ratio 9:1) or low levels of heparin (final heparin concentrations 1 and 2 U/ml). To minimize pre-test blood clot formation or excessive platelet count reduction during blood transport and storage, the experiments with directly heparinized blood were started within 2 hours after blood collection and were completed within 4 hours post blood draw. For the more stable sodium citrate anticoagulated blood, the experiments were started within 4 hours after blood collection and were completed within 8 hours post blood draw. The blood was stored at room temperature before use.

Table 1

Test materials

Material Name (Abbreviation)	Source Information	Test Category
Whole blood (WB)	Healthy human donors, NIH Blood for Research Program	Negative control
Polyurethane (PU) tubing	McMaster-Carr (Robbinsville, NJ, https://www.mcmaster.com), Part #5108K45	Biomaterial
Nylon 6/6 (Nylon) sheet	Plastics International (Eden Prairie, MN, www.plasticsintl.com), Part # Nyn-.031-12-24	Biomaterial
316L Stainless Steel (SS) sheet	Trinity Brand Industries (Burr Ridge, IL). Part # 2316–4	Biomaterial
High Density Polyethylene (HDPE) sheet	Read Plastics (Rockville, MD). Note: No Part# available. This company is no longer in business.	Biomaterial
Buna-N rubber (Buna) sheet	McMaster-Carr (Robbinsville, NJ, https://www.mcmaster.com), Part #8969K72	Positive control
Natural Latex Tubing (latex)	McMaster-Carr (Robbinsville, NJ, https://www.mcmaster.com), Catalog# 5234K931	Positive control
4 mm Soda Lime Glass Beads (glass)	Fisher Scientific (Pittsburgh, PA) Cat#: NC9559146	Positive control

Table 2

Summary of test conditions

Anticoagulant for blood draw	Test Group	Recalcification with $CaCl_2$	Final Heparin conc	Test materials
Sodium Citrate	Citrate	No	N/A	All the materials listed in Table 1 above.
	ReCa+Hep 2U	Yes	2 U/ml	
	ReCa+Hep 3U	Yes	3 U/ml	
Heparin	Direct-Hep 1U	N/A	1 U/ml	HDPE, SS, Buna, and latex
	Direct-Hep 2U	N/A	2 U/ml	

ii) Choice of positive control materials

Our study used the following three materials as positive controls for both platelet and leukocyte count assays: i) Buna, ii) Glass, and iii) Latex. Figure 1 below shows the test results from our study comparing the positive controls with various biomaterials. Our results showed that the platelet counts for all three positive controls were statistically significantly different when compared to the biomaterials. We believe that any of these positive controls could be used for the platelet count assay while following the ASTM F2888-19 standard.

iii) Adjustment of blood anticoagulant concentration

The default blood heparin concentrations recommended in the ASTM F2888-19 standard are: (1) 2 U/ml for the recalcified and heparinized citrate blood and (2) 1 U/ml for directly heparinized blood. Due to inherent donor variability in blood coagulation response to heparin dosage, it is acceptable to use a heparin concentration that is slightly lower or higher (< 1U/ml difference) than the default concentrations to increase test sensitivity or blood stability for blood from some donors, as long as the test results for the negative and positive controls are within the assay validity range stated in Clause 14.2 of the ASTM F2888-19 standard. A detailed discussion on heparin concentration is provided in the DISCUSSION section of Lu et al. 2021 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8490275/)

iv) Assay data

Figure 1 and 2 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8490275/) contains platelet and leukocyte count assay data for different biomaterials, positive controls, and anticoagulation strategies. The data could be used as references by other test labs while developing their own test procedures.

v) Discussion on test sensitivity of the assays

From our data in Figures 1 and 2 below, even though the directly heparinized blood at 1 U/ml had better leukocyte count differentiation between the positive controls and the biomaterials as compared to the recalcified blood, the test sensitivity associated with the leukocyte counts was lower compared to that of the platelet counts for both recalcified blood and directly heparinized blood. Thus, we believe that leukocyte count alone should not be used as an indicator for thrombogenicity evaluation of biomaterials or medical devices.

The study also showed that the assays does not appear to be sensitive enough to distinguish subtle thrombogenicity differences among different biomaterials. Rather, it is suitable only to differentiate the biomaterials from positive controls. The users of this test method should be aware of this limitation while trying to differentiate performance of different medical device materials.

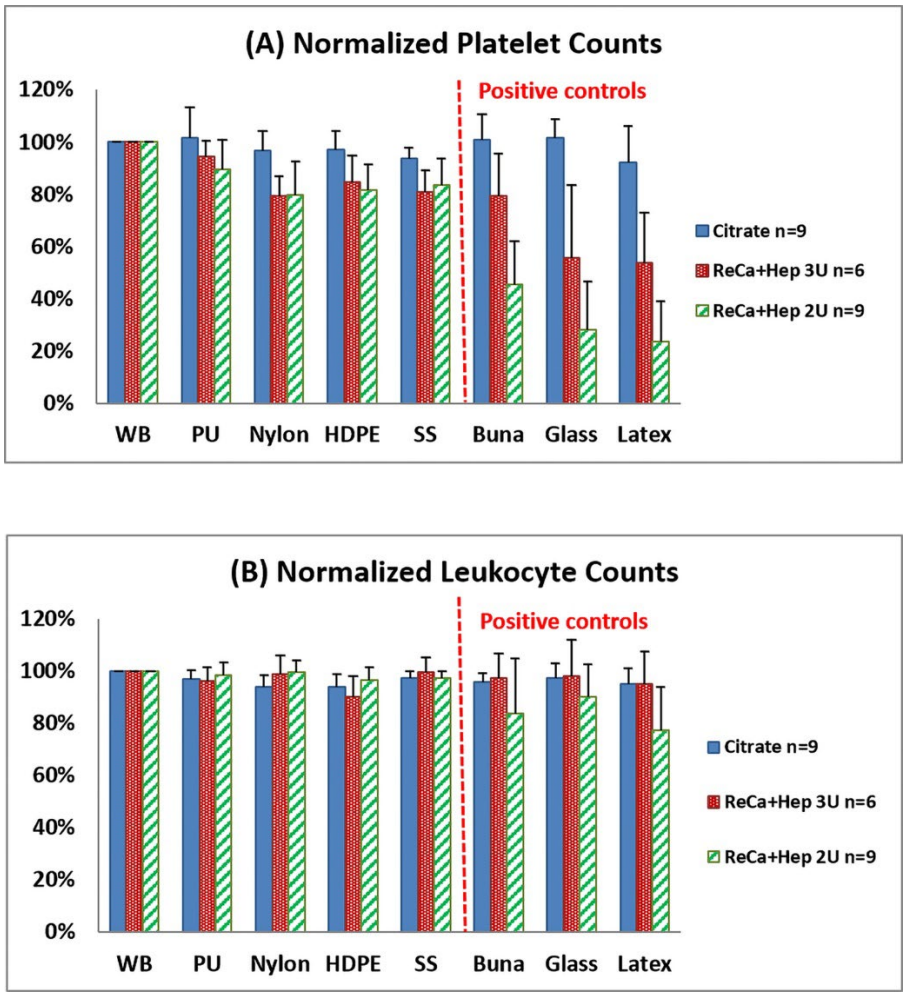
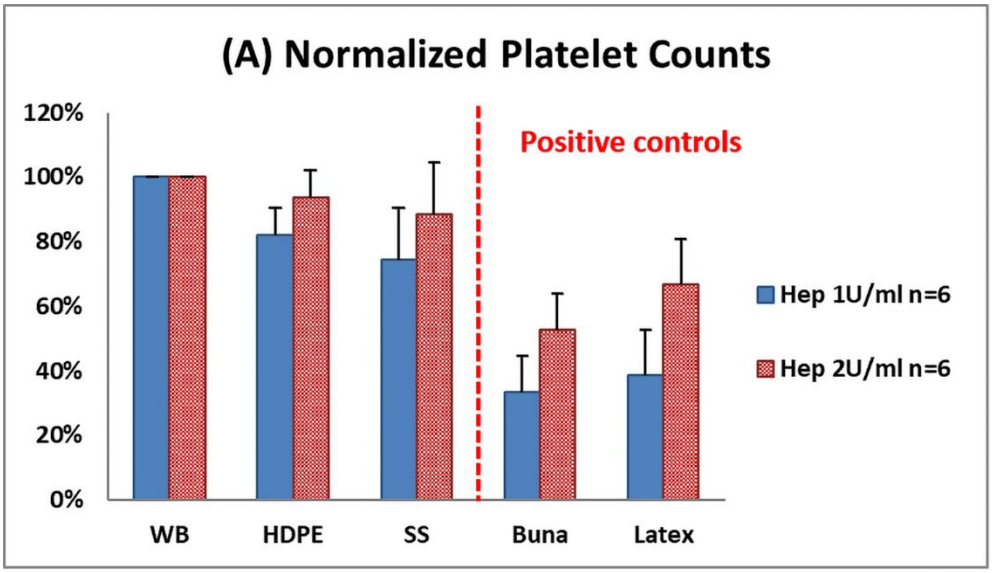


Figure 1: Platelet (A) and leukocyte (B) count results (normalized to the whole blood negative controls for each blood condition) after blood incubation with the test materials for 1 hour. Blood conditions included sodium citrate anticoagulated blood and recalcified citrated blood with added heparin to a final concentration of 2 U/ml (ReCa+Hep 2U) and 3 U/ml (ReCa+Hep 3U).



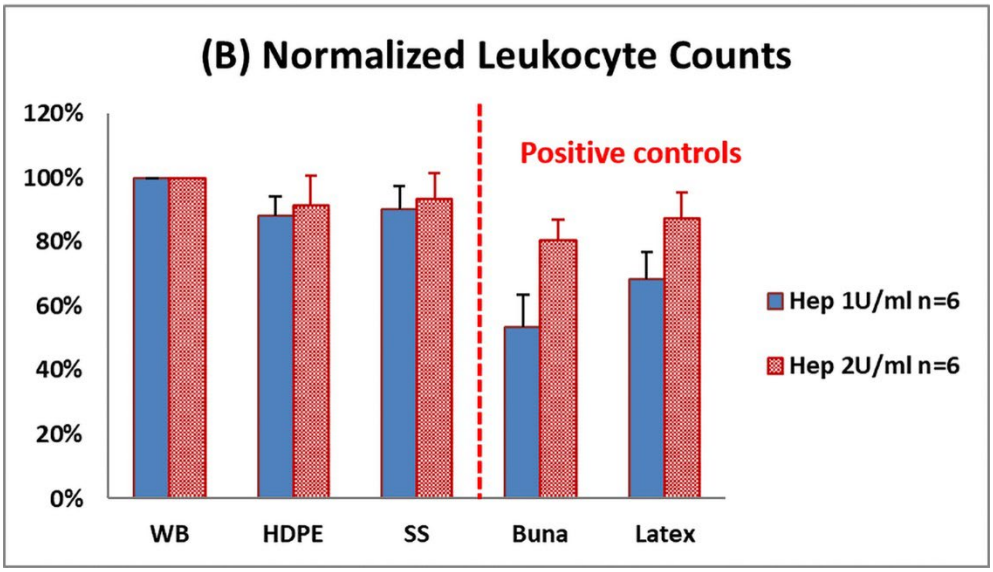


Figure 2: Platelet (A) and leukocyte (B) count results (normalized to the whole blood negative controls for each blood condition) after blood incubation with the test materials for 1 hour. Tested with directly heparinized blood to concentrations of 1 U/ml and 2 U/ml.