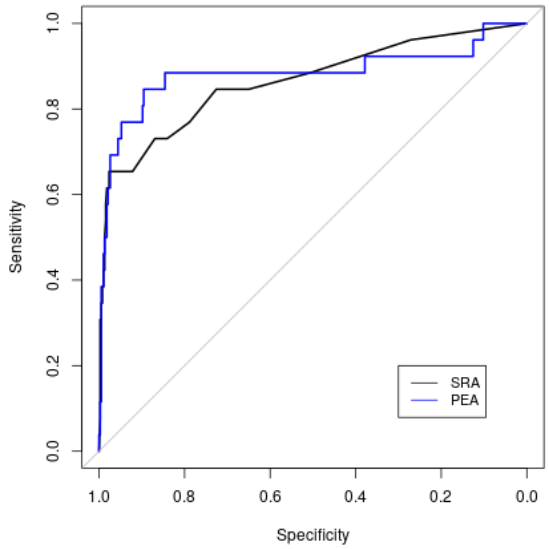
Supplementary Materials:

Figures



	AUC	Lower CI: 95%	Upper CI: 95%
PEA	0.8832	0.7838	0.9826
SRA	0.8644	0.7736	0.9552

Figure S1. PEA accuracy. Receiver operator curve (ROC) testing for the SRA and PEA was performed with HIT "indeterminates" considered *disease-positive*. Area under the curve (AUC) estimates with confidence intervals are also presented.

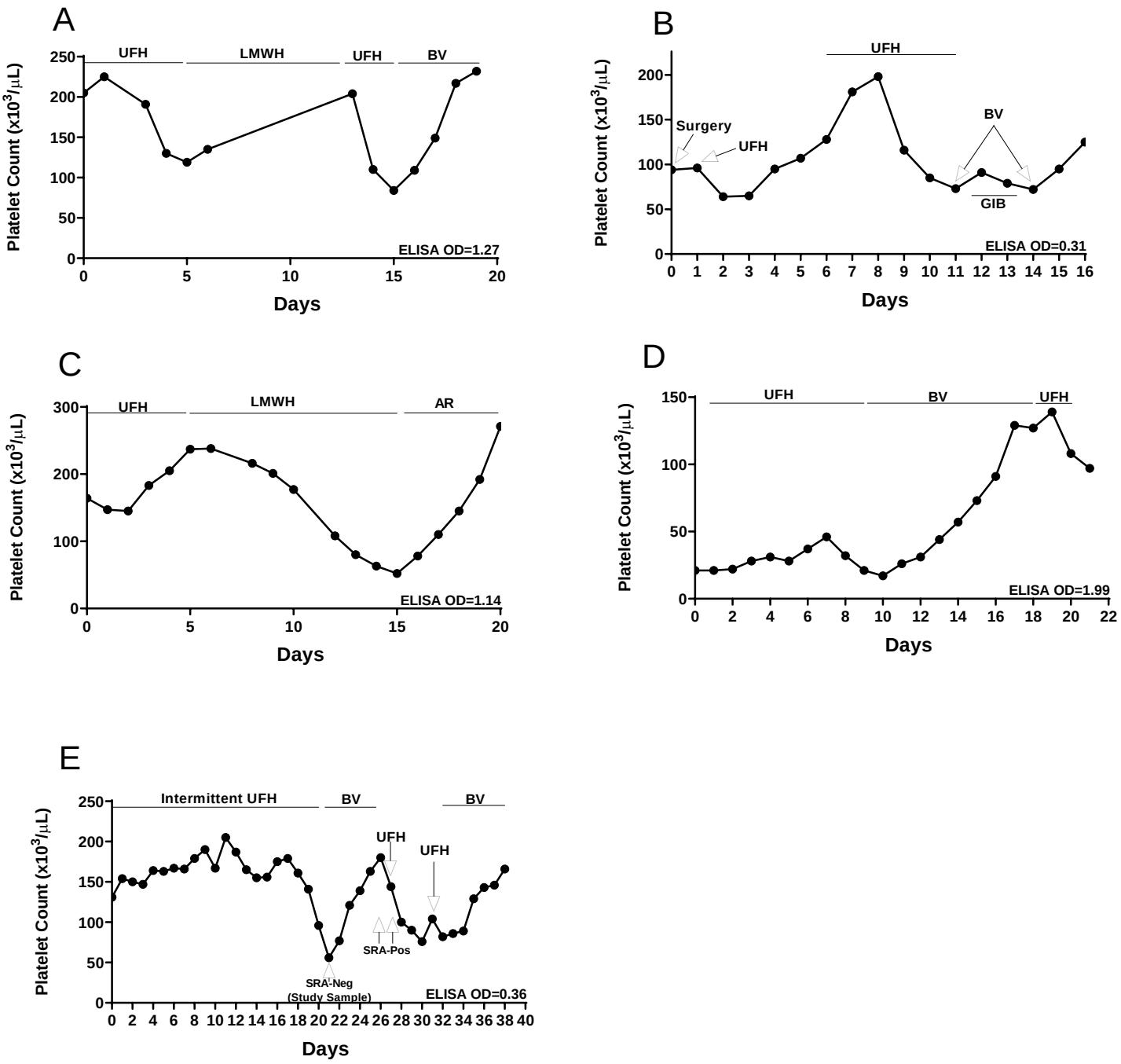


Figure S2. PEA+/SRA- patients: Platelet counts in relation to heparin treatment. HIT ELISA OD is indicated on the bottom right of each graph. Platelet counts are indicated by closed circles. If multiple platelet counts were obtained on a given day, the mean count was calculated and utilized for graphing. Patients presented is **S2A, B, C, D** and **E** correspond to patients 1, 2, 3, 4 and 5, respectively from **Table 3B**. UFH- Unfractionated heparin; LMWH- Low molecular weight heparin; BV- Bivalirudin; AR-Argatroban; GIB-Gastrointestinal bleeding; ELISA-Enzyme-linked immunosorbent assay; OD-Optical Density.

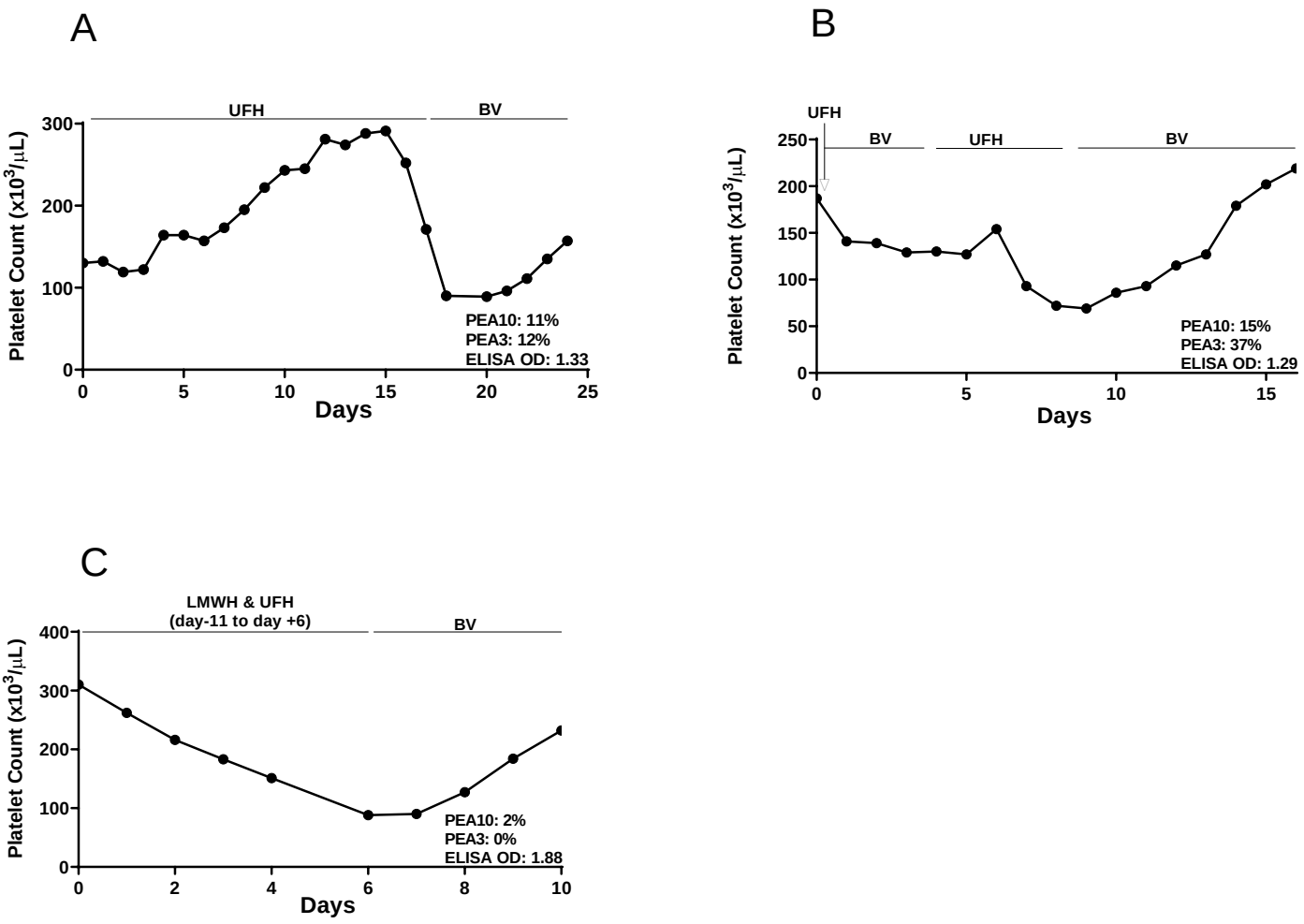
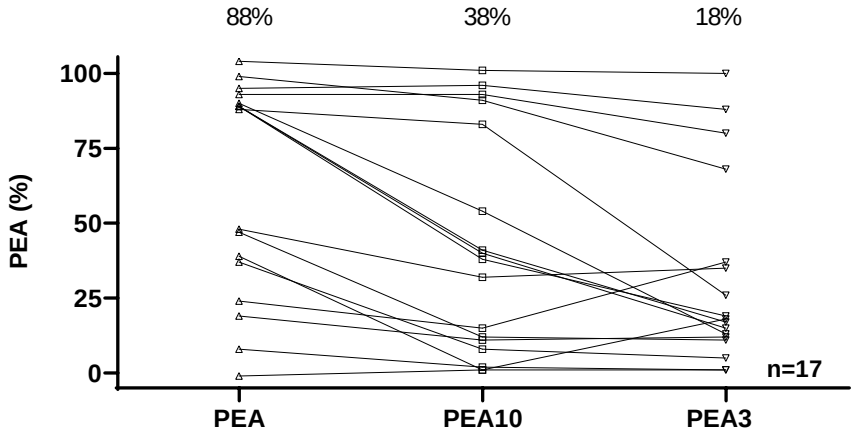
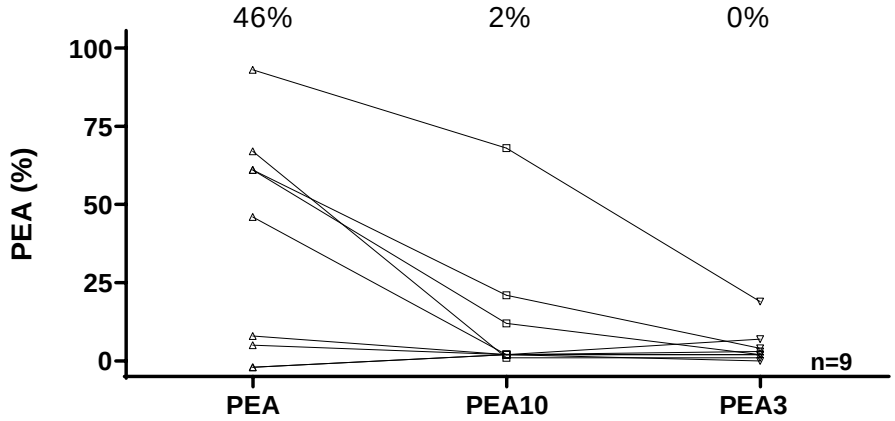


Figure S3. SRA+/PEA- patients: Platelet counts in relation to heparin treatment. HIT ELISA OD, PEA10 and PEA3 results are provided on the bottom right of each graph. Platelet counts are indicated by closed circles. If multiple platelet counts were obtained on a given day, the mean count was calculated and utilized for graphing. Patients presented in **A, B** and **C** correspond to patients 1, 2 and 3, respectively from **Table 3C**. UFH-Unfractionated heparin; LMWH- Low molecular weight heparin; BV- Bivalirudin; ELISA-Enzyme-linked immunosorbent assay; OD-Optical Density.

A



B



C

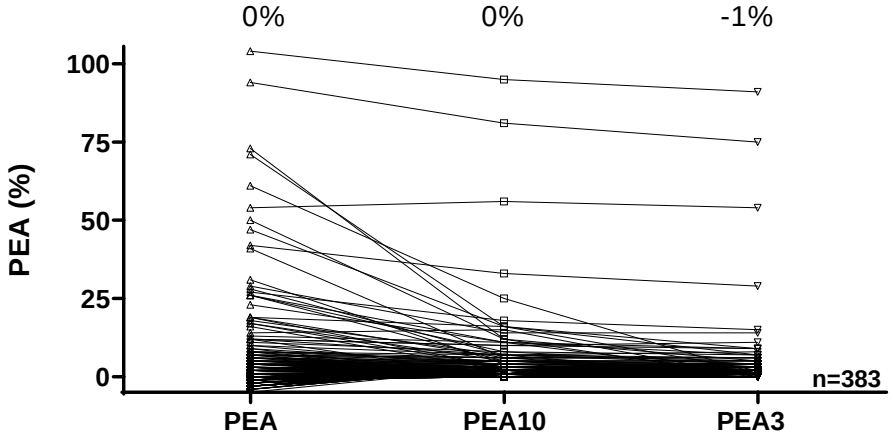
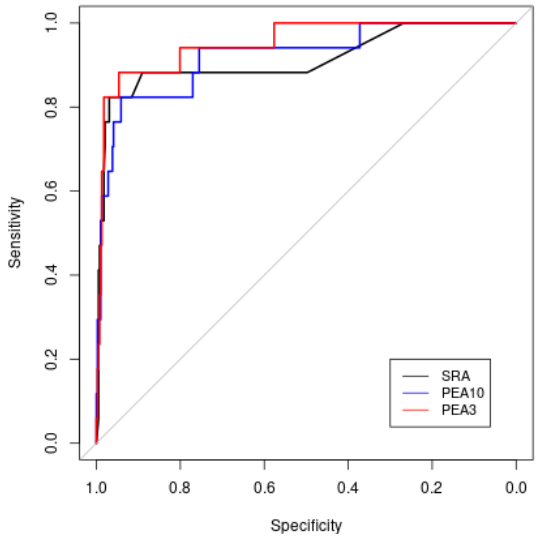


Figure S4. PEA test results. PEA results at the "standard" and lower concentrations of PF4 are shown in HIT-positive (A), HIT-indeterminate (B) and HIT-negative (C) samples. Open triangles, squares and inverted triangles represent PEA, PEA10 and PEA3 results, respectively. Median results are listed on top of each data set.



	AUC	Lower CI: 95%	Upper CI: 95%
PEA10	0.9218	0.8449	0.9987
PEA3	0.9521	0.9002	1.000
SRA	0.9124	0.8166	1.000

Figure S5. Test accuracy of PEA assays performed with lower concentrations of PF4. Receiver operator curve (ROC) testing for the SRA and PEA assays performed at lower PF4 concentrations (PEA10, 10ug/mL PF4 ; PEA3, 3ug/mL PF4) was performed. Area under the curve (AUC) estimates with confidence intervals are also presented. The SRA AUC is provided for comparison.

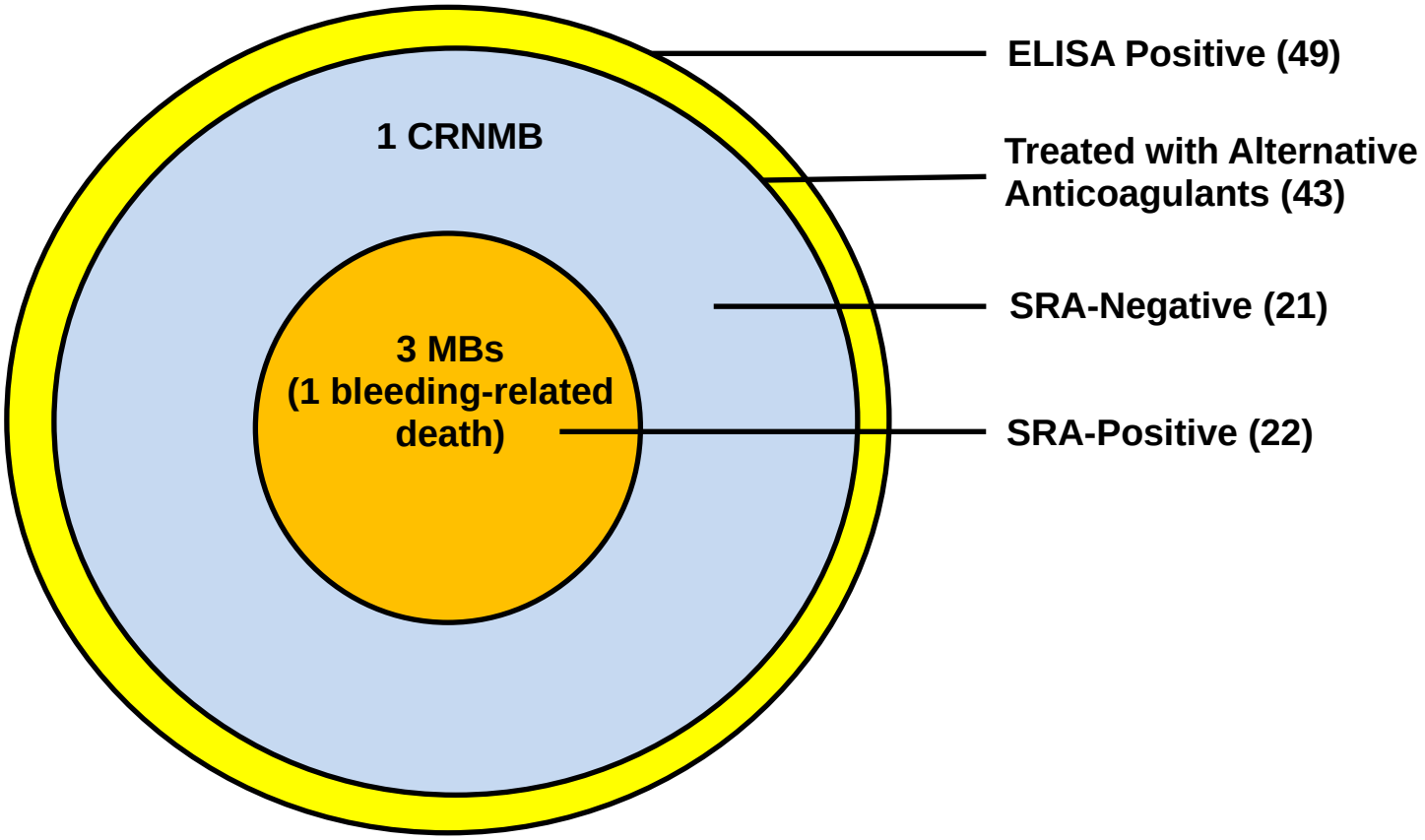


Figure S6. Excessive use of alternative anticoagulants in HIT. Alternative anticoagulant use and bleeding events in HIT ELISA-positive patients from the study. CRNMB- Clinically-relevant non-major bleeding; MB- Major bleeding.

Tables

Table S1. Concordance between the PEA/SRA and HIT ELISA.

		HIT ELISA			
		Negative	**Positive**	**Positive Concordance**	**Negative Concordance**
SRA	**Negative**	356	27	0.449	0.989
	Positive	4	22		
PEA	**Negative**	356	25	0.490	0.989
	Positive	4	24		

Table S2. HIT-positive patients with negative results in both PEA and SRA.

								HIT diagnosis	
N	**Age**	**Sex**	**4Ts Score**	**Thrombosis***	**PEA**	**SRA**	**ELISA OD**	**Predefined[1]**	**Full Review[2]**
1	72	M	8	2	-1	8	1.06	Positive	Positive
2	71	F	6	1	8	1	1.35	Positive	Negative[3]

[1]Predefined criteria included ELISA OD and 4Ts scores calculated at the time of HIT suspicion (Table 1A). [2] HIT was adjudicated based on integrating 4Ts score calculated at the time of HIT suspicion as well as follow up information such as platelet trending upon heparin cessation and upon heparin re-exposure (if re-exposure occurred). * "Yes", "Maybe" and "No" for thrombosis correspond to 4Ts scores of 2, 1 and 0, respectively for thrombosis. [3] A second SRA was obtained for clinical purposes and was also negative. Heparin was restarted with no adverse sequelae.

Table S3. Activating antibodies in high, intermediate and low 4Ts scored patients.

	SRA-Positive, n (% of Total)	PEA-Positive, n (% of Total)	Total (n)
High 4Ts Score (6-8)	11 (41%)	11 (41%)	27
Intermediate 4Ts Score (4-5)	10 (10%)	12 (12%)	98
Low 4Ts Score (0-3)	5 (2%)	5 (2%)	284

Table S4. SRA-positive antibodies in patients classified as HIT-positive, indeterminate and negative.

	SRA≥20%, n (% of Total)	Total (n)
HIT-Positive	14 (82%)	17
HIT-Indeterminate	3 (33%)	9
HIT-Negative	9 (2%)	383

Methods

PEA/SRA testing

The PEA was performed as described previously[1,2]. Briefly, pooled platelets isolated from three blood group O donors was used in testing. Prostaglandin E1 (50 µg/mL) was added to platelet-rich plasma (PRP) and centrifuged at 150 x g for 15 minutes. The supernatant was then centrifuged at 1,000 x g for 15 minutes to pellet platelets. The platelet button was re-suspended in phosphate-buffered isotonic saline (pH 7.4)-1% bovine serum albumin. These washed normal donor platelets (1×10^6) were first treated for 20 minutes at room temperature with PF4 (37.5, 12.5 or 3.75 µg/mL) in a total volume of 40 µL. Ten microliters of patient sample was then added and the mixture was incubated for 1 hour at room temperature giving a final PF4 concentration of 30µg/mL (PEA), 10µg/mL (PEA10) or 3 µg/mL (PEA3). After addition of labeled anti-P-selectin (Monoclonal antibody 424.2, Versiti Wisconsin) and anti-GPIIb (Monoclonal antibody 290.5, Versiti Wisconsin) antibodies, platelet events were gated by GPIIb positivity in flow cytometry, and P-selectin expression (median fluorescence intensity, MFI) was recorded. In addition to a normal sample "calibrator," known positive and negative patient samples were included in each run. Maximum P-selectin expression (100%) was measured by treating platelets with thrombin receptor-activating peptide (TRAP, 25 ug/mL). Results were expressed as the percentage of maximum P-selectin expression corrected for background signal obtained with normal sample as follows:

Percent activation= [(Sample MFI-Normal serum MFI)/(TRAP MFI-Normal serum MFI)] x 100.

The SRA was performed by the Versiti Wisconsin's CLIA-approved laboratory as previously described[3].

References:

1. Padmanabhan A, Jones CG, Curtis BR, et al. A Novel PF4-Dependent Platelet Activation Assay Identifies Patients Likely to Have Heparin-Induced Thrombocytopenia/Thrombosis. Chest 2016;150:506-15.
2. Padmanabhan A, Jones CG, Pechauer SM, et al. IVIg for Treatment of Severe Refractory Heparin-Induced Thrombocytopenia. Chest 2017;152:478-85.
3. McFarland J, Lochowicz A, Aster R, Chappell B, Curtis B. Improving the specificity of the PF4 ELISA in diagnosing heparin-induced thrombocytopenia. Am J Hematol 2012;87:776-81.