Impact of recalcification on procoagulant COAT platelet generation in PRP samples with low platelet counts

Abstract category : Hemostasis, transfusion medicine, vascular, laboratory medicine

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INTRODUCTION

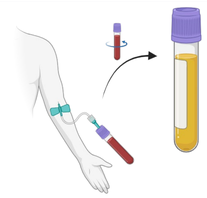
Assessment of platelet procoagulant function by flow cytometry is an increasingly recognized aspect of diagnosing platelet disorders. Induction and accurate detection of phosphatidylserine exposure on platelets' surface requires proper recalcification of citrated blood samples, particularly in thrombocytopenic patients, where residual citrate can limit extracellular calcium availability. Recalcification of thrombocytopenic blood samples is crucial for procoagulant platelets generation and detection.

AIM

The objective of this project was to define optimal recalcification conditions for reliable measurement of procoagulant (**COAT**) platelets in platelet-rich plasma (**PRP**) samples with low platelets counts.

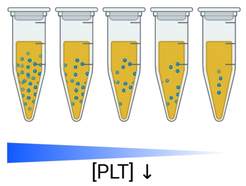
METHODS

1



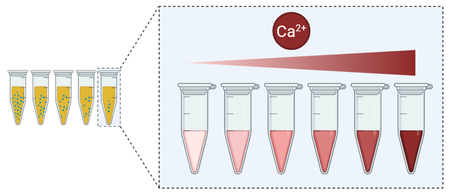
Fresh PRP and PPP from healthy donors

2



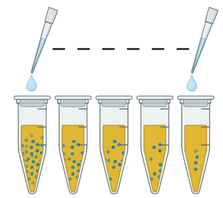
PRP diluted with autologous PPP (160 → 15 G/L)

3



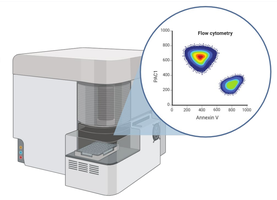
Each PLT dilution was spiked with increasing [Ca^{2+}] (0 [HEPES], 1, 2, 3, 5, 7, 10, 15 mM).

4



Stimulated with convulxin (**CVX**) + thrombin (**THR**).

5



Quantification of procoagulant platelet formation vs. platelet count and Ca^{2+} level by flow cytometry. (Accuri C6)

RESULTS

Figure 1

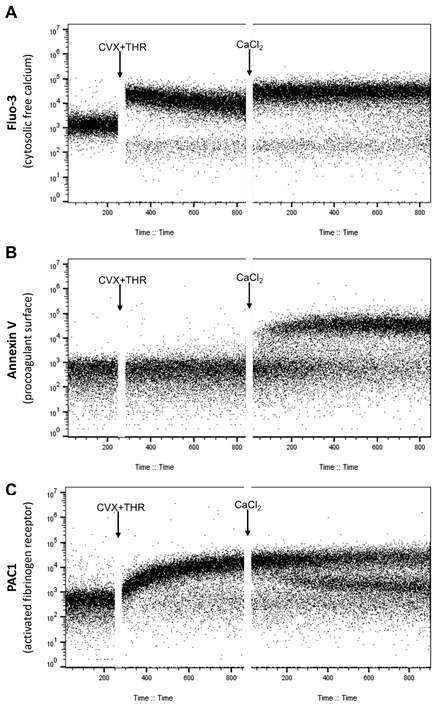
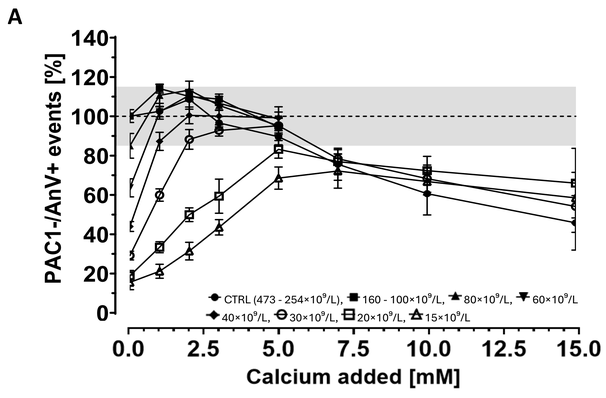
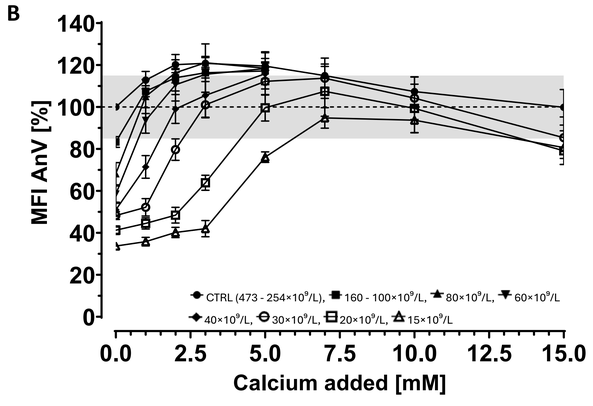


Figure 2





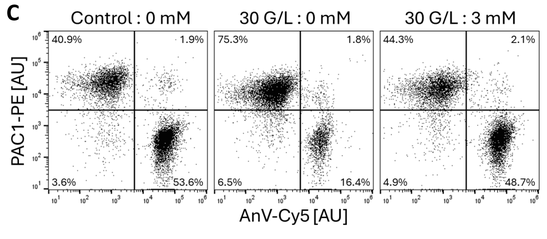


Figure 1. Extracellular calcium is required to sustain intracellular calcium elevation and promote procoagulant platelet formation. Platelets were activated with CVX+THR for 10 minutes and 2mM calcium was then added. **(A)** Intracellular calcium kinetics monitored by Fluo-3 fluorescence, **(B)** phosphatidylserine exposure assessed by annexin V binding and **(C)** fibrinogen receptor activation monitored by PAC1 binding over time.

Figure 2. Procoagulant COAT platelet generation is artifactually impaired in samples with low platelet count and can be restored with extracellular calcium spiking. (A) Generation of PAC1-/AnV+ events (procoagulant COAT platelets) and **(B)** Median fluorescence intensity (MFI) of annexin V (**AnV**) binding were expressed relatively to the control. **(C)** Addition of extracellular calcium to the 30 G/L simulated PRP restores both procoagulant COAT platelet generation and the loss of AnV MFI compared to control.

CONCLUSIONS

For PRP samples with platelet counts ≥100 G/L, no additional calcium beyond buffer levels was required. For platelet counts between 30–80 G/L, supplementation with 3 mM calcium restored procoagulant platelet generation within 85–115% of undiluted PRP control levels. Counts of 20–30 G/L required 5 mM calcium. Of note, higher calcium concentrations (>5 mM) impaired platelet function, highlighting the need to avoid excessive recalcification. Optimized recalcification prevents underestimation of procoagulant platelet potential in thrombocytopenic samples. This practical approach addresses a key gap identified by the ISTH SSC and helps laboratories to ensure reliable platelet function testing in PRP samples from thrombocytopenic patients.

CONTACT INFORMATION

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