

## HIGHLIGHTED PLATELET FUNCTION ASSAY

### Functional and Phenotypic Microparticle Analysis

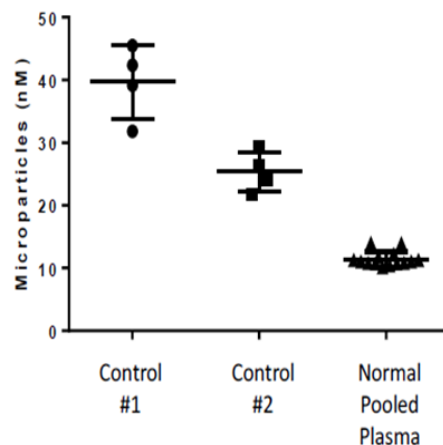
Circulating stimuli, including inflammatory cytokines and some therapeutics, activate platelets and endothelial cells. Measurement of circulating microparticles serves as an indicator of in vivo vascular function, and in clinical studies is used to identify risk of thrombosis and bleeding.

#### Microparticle Procoagulant Activity- ELISA Detection

Activated platelets and endothelial cells release microparticles with exposed phosphatidyl-serine (PS), a procoagulant stimulus for thrombin generation. Procoagulant plasma microparticles are quantitated using an ELISA platform in which PS-presenting microparticles are captured on an annexin V-coated microplate. In vitro thrombin generation and catalytic activity are then measured as a readout for total microparticle concentration.

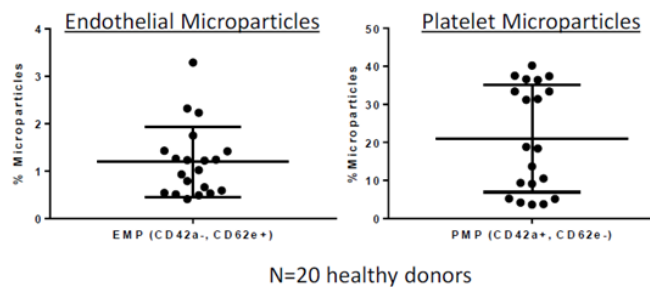
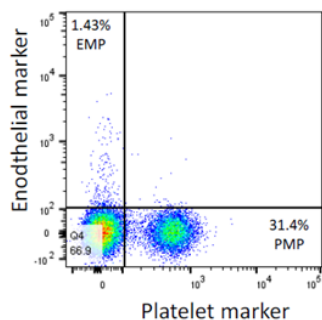
#### Microparticle Phenotyping- Flow Cytometry Detection

Cell origin and microparticle phenotype are established utilizing antibody labeling of cell-type specific proteins on the microparticle surface. In normal donors, endothelial microparticle (EMP) levels are low (<5%), and are differentiated from other circulating microparticles, the majority of which are platelet-derived, by labeling E-Selectin (CD62e), a cell adhesion molecule expressed exclusively by endothelial cells



Procoagulant microparticles were quantitated using ELISA for two controls (n=4) and pooled plasma from normal donors (n=15).

Microparticle phenotype and percentages, relative to total microparticles, were determined for normal donors (n=20) using flow cytometry.



Platelet glycoprotein IX (CD42a) is a platelet-specific membrane protein utilized to label platelet microparticles (PMP). Labeled microparticles are analyzed by flow cytometry, and results are reported as percent EMP or PMP, relative to total microparticle count.

CirQuest offers functional and phenotypic microparticle analysis of pre-clinical and clinical samples, and will customize for specific membrane antigens. Plasma microparticles are sensitive to sample handling and storage conditions. Our biostorage and central lab capabilities ensure rigorous quality control and documented reproducibility.