VetStem

PRECISEPRP™

Uniformity in Platelet Rich Plasma Treatment

Platelet rich plasma has been used in the practice of veterinary regenerative medicine for over twenty years. Variation in PRP occurs due to donor source, platelet function of the donor, platelet count of donor, contamination with other cell types and bacteria, activation level at the time of use, administration route and etiology of the lesion treated. Standardization of PRP provides the industry with the opportunity to evaluate PRP in specific disease indications without "noisy" variability between products.

PrecisePRP™ is designed through our patent pending process to minimize many of these variations by using thoroughly screened donors in consistent, GMP compliant processes within a controlled manufacturing environment.

Table 1 PrecisePRP™ is tested for the following characteristics to insure uniformity with every batch.

Characteristic	Assay	Acceptance Criteria	Comments
Identity	Flow cytometric assay	CD61 >/= 40%	Presence of CD 61 supports the presence of platelets
Identity	Flow cytometric assay	CD9 >/= 40%	Presence of CD 9 supports the presence of platelets
Identity	Flow cytometric assay	>/= 75% of platelets are between 0.2µM to 5.0µM	Supports the presence of intact platelets
Purity	Residual moisture	</= 2.0%	Karl Fischer method indicates solid phase stability
Strength	Platelet count by impedance counter	500,000 platelets/µl +/- 100,000 platelets/µL	Total platelet dose relates to final product activity
Safety	USP 71 Sterility	Negative for aerobes, anaerobes and fungus	
Safety	USP 63 Mycoplasma	Negative for mycoplasma	
Safety	USP 85 Endotoxin	< 2EU/mL	Supports low endotoxin level for intra-articular use
Potency	ELISA PDGF-BB	Reported	Relates to final product activity

Uniformity of total platelet dose delivered and a confirmation of negative status for bacterial, mycoplasma, and fungal contamination every time provide you with confidence in drug delivery.

VetStem

IN VITRO ASSESSMENT OF POOLED PLATELET PRODUCT

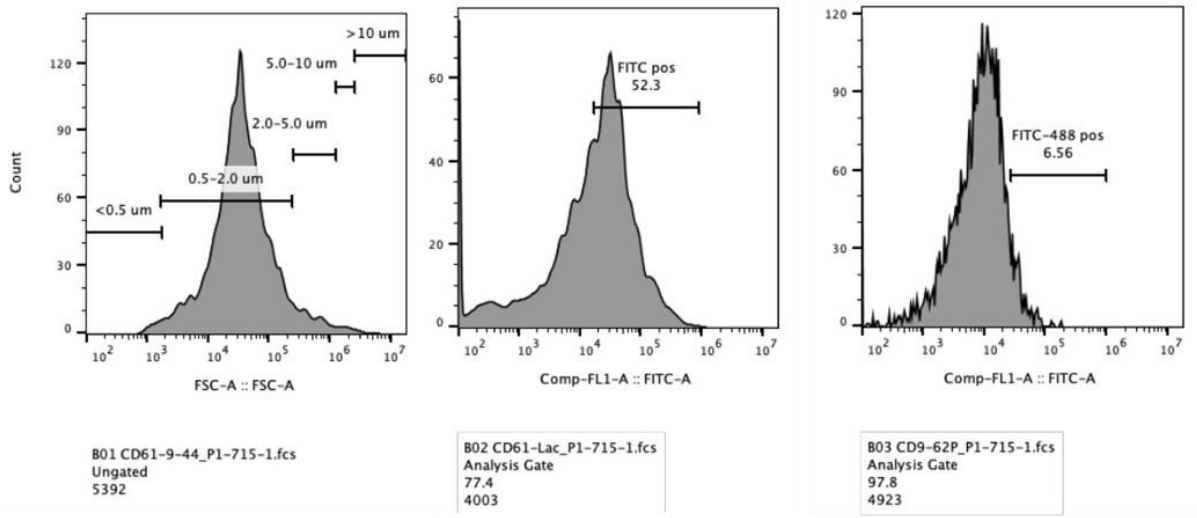
Study Overview:
Flow cytometric assays were performed to characterize a lyophilized platelet product. This pilot study investigated 6 samples (each reconstituted in 8 mL) from three separate pilot runs. Aliquots of each vial were labeled with a panel of markers summarized in the table below. Analyses include percentage of positive events within a gate defined by size/scatter properties, and particle size within gates defined by calibrated microspheres. Antibody-conjugated beads and aliquots of frozen/thawed canine platelet rich plasma were used for compensation and labeling controls, and isotype antibodies were also used to set positive/negative threshold boundaries.

Results:
Individual cytometry plots are displayed below. Three labeling reactions were performed for each sample: 3 constitutive markers (CD61-PE, CD9-APC, CD44-FITC); constitutive platelet membrane marker & phosphatidylserine (PS) marker (CD61-PE & lactadherin-FITC); constitutive platelet membrane marker & P selectin marker (CD9-APC & CD62PDY488.) An acquisition forward scatter threshold of 8,000 was used to allow detection of small (< 0.5 µM) events and minimize machine noise and debris. 40 -50% of lyo-particles expressed both the platelet membrane markers CD61 & CD9, with relatively few (< 1%) particles expressing leukocyte marker CD44 confirming a leucoreduced platelet product. Phosphatidylserine externalization (40 to 50%) was another consistent feature supporting a platelet status of “primed for activation.” P-selectin remained low indicating that granules had not “dumped.” Particle size appeared uniform with predominant size in most samples falling within the 0.5 to 2 micron gate.

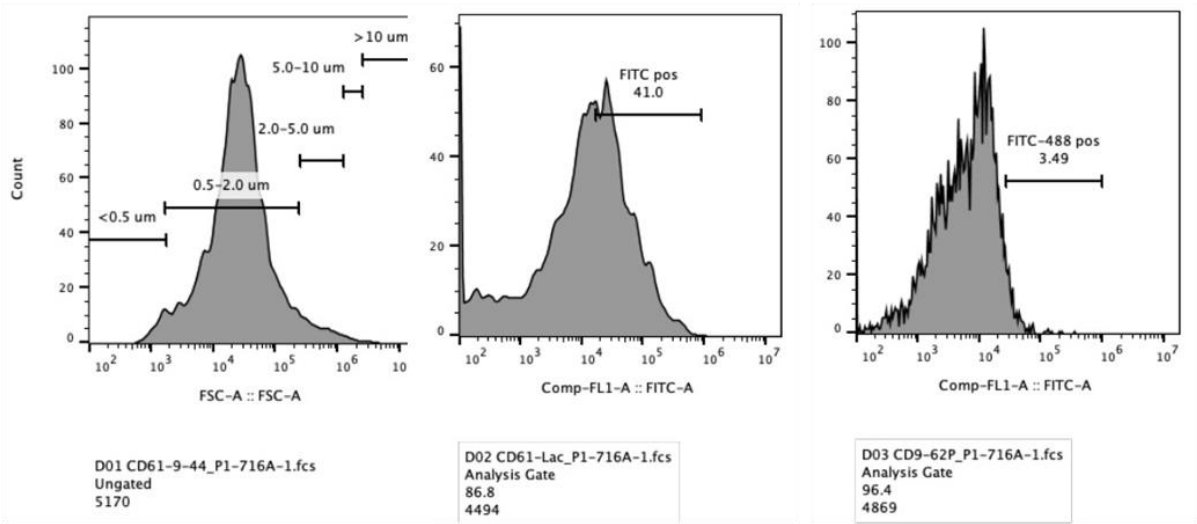
Conclusions:
Evaluation of three separate pilot manufacture runs support the uniformity of PrecisePRP™ post rehydration relating to particle sizing, leucoreduction, activation status and membrane marker identity. With this type of uniformity, this PRP offers the opportunity to evaluate treatment and outcome without the “noise” of autologous PRP production caused by donor source, platelet function of the donor and activation level at the time of use. This should also improve consistency of clinical outcome effect by standardizing the quality and dose.

Figure 1 (next page): The following graphs demonstrate the uniformity of platelet identity, size, and activation level across pilots one, two, and three. The first graph for each pilot shows platelet identity and size. The second and third graph in each pilot demonstrates that the great majority of the platelets contained in each sample are primed for activation but have not yet released their granular contents.

PILOT 1



PILOT 2



PILOT 3