

PRODUCING ACCURATE PLATELET COUNTS FOR PLATELET RICH PLASMA: VALIDATION OF A HEMATOLOGY ANALYZER AND PREPARATION TECHNIQUES FOR COUNTING

Jennifer E. Woodell-May, Ph.D.^{1,3}, Dayna N. Ridderman¹, Matthew J. Swift, M.S.², and
Joel Higgins, B.S.²

1-Biomet, Inc. (Warsaw, IN)

2-Cell Factor Technologies, Inc. (Warsaw, IN),

3-Corresponding Author: Phone: (574)-372-1757 Fax: (574)-268-2742

Email: jennifer.woodell@biometmail.com

Accepted by Journal of Craniofacial Surgery January 24th, 2005

ABSTRACT

Platelet rich plasma (PRP) has been shown to clinically accelerate healing of both soft and hard tissues. As a result, it has gained increasing popularity. However, the clinical effectiveness of each type of PRP preparation method can vary in technique and efficiency, and current methods to evaluate the platelet concentration efficiency of PRP systems have several limitations. Therefore, the purpose of this study was to validate an automated hematology analyzer, the Cell-Dyn 3700, in order to accurately count platelets in concentration ranges of approximately 2-5,000,000 platelets/ μ L. PRP platelets were counted via a manual counting method and on the Cell-Dyn 3700 and the statistical evaluation indicated no difference between the groups ($p > 0.05$). Dilution of the PRP was not required and accurate platelet counts could be achieved up to platelet concentrations of 4,800,000 platelets/ μ L. PRPs must be resuspended on a rocker for at least five minutes before platelet counts, and the entire PRP sample must be resuspended to allow for equal distribution of platelets before counting. Using the validated Cell-Dyn 3700, a platelet concentrate system used to prepare 153 PRPs. The baseline whole blood platelet concentration (328,000 platelets/ μ L \pm 69,000 platelets/ μ L) and the average PRP samples (2,645,000 platelets/ μ L \pm 680,000 platelets/ μ L) were compared, resulting in an 8 fold increase in concentration and an average platelet percent recovery of approximately 76%. Automated hematology analyzers can be used to accurately count platelets in PRP given the system has been validated appropriately and the PRP samples are prepared properly in order to provide adequate platelet suspension.

KEYWORDS: hematology analyzer, platelet concentrate, platelet rich plasma, platelet counts, Cell-Dyn

INTRODUCTION

Platelet rich plasma (PRP) is a concentrated platelet product that can be produced from whole blood through multiple commercially available systems, resulting in varying levels of platelet concentration (1,2). Platelets play a crucial role in the signaling cascade of normal wound healing. Activated platelets release the contents of their α -granules resulting in a deposition of powerful growth factors such as platelet derived growth factor (PDGF), transforming growth factor β (TGF- β), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) (3-5). PRP has been used in many different clinical applications, demonstrating the effectiveness and importance of the product for a variety of medical procedures. For example, percutaneous application of PRP to patients with severe lateral epicondylitis, or tennis elbow, resulted in improved elbow function and reduced pain (6). Early maturation of bony fusion was observed when platelet concentrate was used during lumbar spinal fusions (7). Chronic diabetic foot ulcers treated with PRP achieved increased healing rates compared to the control group receiving standard care (8). Studies by Bhanot *et al.* show decreased formation of hematoma and seroma, decreased postoperative swelling, and improved healing time for plastic surgeries that included PRP in the treatment (9). Further, during dental surgeries, the use of PRP has improved bone regeneration around implants (10).

There are many different systems on the market that produce PRP, with a high variability in the final product's characteristics, including platelet concentration, volume of output, and fibrinogen concentration. The goal of each of the systems is to concentrate platelets in an easily extractable product. Marx suggests that the goal of a PRP is to concentrate platelets 3-5 times over the baseline platelet counts, or at a

concentration of at least 1,000,000 platelets/ μL (11). Before conducting PRP efficacy studies, or before trying to draw conclusions on the number of platelets required for each surgical application, it is necessary to be able to accurately determine how many platelets, and consequently the concentration of growth factors, that are applied to the surgical site. However, a twofold problem exists when determining the success of the systems to effectively concentrate the platelets. First, each device outputs a differently concentrated product, and second, the methods to quantify efficiency of concentration do not appear to have been validated.

Automated hematology analyzers are routinely used for complete blood counts (CBCs) and have been validated to count elements of whole blood, including platelets (12). These validations, however, are not sufficient when counting platelets in PRP for several reasons. Marx cited an instance where a Coulter Counter counted each clump of platelets each as one single platelet, giving an falsely low platelet count (13). However, some hematology analyzer systems are equipped to flag counts with suspected platelet clumping, avoiding this problem. Additionally, the manufacturers of each of the analyzers determine an upper limit of the linear range of platelets that can be counted, which PRP can often exceed. Furthermore, platelets in PRPs do not stay suspended in solution and settle within seconds. This phenomenon could be due to the reduced red blood cell (RBC) number. As a result of the decreased hematocrit, PRPs are optically lighter in color and can cause errors, such as incomplete aspiration, indicating that the system did not aspirate enough blood to make an accurate count. Given these limitations to counting PRP with hematology analyzers, effort must be made to solve these issues in order to count platelets accurately. The aim of this study is to validate a platelet counting method for PRPs using an automated hematology analyzer.

MATERIALS AND METHODS

PRP was produced using a commercially available system (GPSTM II system, Cell Factor Technologies, Warsaw, IN) according to the product insert instructions. Briefly, 60 cc of whole, anticoagulated blood was inserted into the PRP disposable. The disposable was then centrifuged for 15 minutes at 3,500 rpm. During the centrifugation step, a tuned-density buoy floats in-between the RBCs

and the buffy coat, which contains the white blood cells and platelets. A second buoy moves from the top of the disposable, separating the buffy coat and 6 cc of plasma from the rest of the blood plasma. Following centrifugation, the excess plasma was removed, and the platelets were resuspended in the PRP by vigorously shaking the disposable for 30 seconds. The PRP was transferred to 13 x 100 mm Elkay tubes and placed on an Ames Aliquot Mixer (Model 4651, Ames Company, Elkhart, IN) before counting.

For each of the following studies, PRPs were made with either bovine blood (Lampire Biological Laboratories, Pipersville, PA) or fresh human blood. Bovine blood was used in many of the tests because it is readily available in large quantities. Therefore, many of the studies were done with bovine blood, but then tested with smaller human sample sizes to ensure the tests are adequate for both species. Both blood sources were anticoagulated with a citrate-based anticoagulant. Stored bovine blood was determined to be viable for platelet counts up to 7 days after harvesting (data not shown).

I. Validation of platelet counts on a hematology analyzer

The Cell-Dyn 3700 (Abbott Labs, Dallas, TX) hematology analyzer was the system selected to be used in this study because it has a high linear platelet range ($2,000 \times 10^3$ platelets/ μL , per the manufacturer) and includes a veterinary package, which is a software package that allows multiple species to be counted. To validate platelet counts on the hematology analyzer, manual counts were compared to the Cell-Dyn 3700 counts. PRP samples for optical manual counts were prepared in the Unopette microcollection system (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) in order to lyse the RBC, and then counted with a hemacytometer (Model 1483, Hausser Scientific, Horsham, PA), using an Olympus BX51 transmission-light microscope at 400x magnification. While counting with the Cell-Dyn 3700, the samples were kept on the rocker before sampling to ensure they were completely mixed and the platelets were evenly distributed.

The number of samples required for statistical significance when comparing manual and hematology analyzer counts was determined from a pilot study (data not shown). Briefly, one PRP sample was manually counted 10 times, and the standard deviation was determined to be 133×10^3 platelets/ μL . Using the sample number equation, $n = [(Z_{\alpha/2} \sigma) / E]^2$, where E is the maximum error (determined to be $\pm 100,000$

platelets), σ is the standard deviation, and $Z_{\alpha/2}$ is the Z statistic determined by the level of confidence (α), the sample size was calculated to be 21 for a 95% confidence level. Therefore, an isolated PRP was counted 21 times manually and with the Cell-Dyn 3700 in order to validate the hematology analyzer for each PRP preparation. This was repeated for three different PRP preparations.

II. Increase in linear range for the hematology analyzer

The Cell-Dyn 3700 has a manufacturer-validated linear range limit for platelets of $2,000 \times 10^3$ platelets/ μL . In order to increase this range, PRPs were counted on the Cell-Dyn 3700 with platelet counts of approximately $4,000 \times 10^3$ platelets/ μL . This concentration was selected because it was well above the expected PRP concentrations typically seen clinically. PRPs were created with bovine blood using the GPSTM II. In order to increase the platelet concentration up to approximately $4,000 \times 10^3$ platelets/ μL , 1-2 mL of plasma was removed from the buffy coat fraction before resuspending the platelets. This resulted in the buffy coat being suspended in less than 10% of the initial starting volume, concentrating the platelets well above what is normally expected clinically. Each concentrated PRP was divided into three samples, one left undiluted, one diluted 1:2 times (50% of initial concentration) with phosphate buffered saline (PBS, pH 7.4), and one was diluted 1:4 times (25% of initial concentration) with PBS. All samples were then counted in triplicate on the Cell-Dyn 3700.

III. Validation of platelet counts with citrate anticoagulated blood

The Cell-Dyn 3700 is only validated by the manufacturer for counting platelets in blood anticoagulated with EDTA, as this is the most common anticoagulant used for clinical laboratory blood work. However, citrate based anticoagulant is the preferred anticoagulant when producing PRP because it increases platelet viability (14). Whole blood from three human volunteers was drawn into EDTA-coated Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and uncoated Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) with 0.8 mL ACD-A (Anticoagulant Citrate Dextrose Solution, Solution A, Citra Anticoagulant, Inc., Braintree, MA). The dilution factor for the ACD-A anticoagulated blood was 1.11. Cell counts were compared between the two anticoagulated blood groups (n=3 per sample). Statistical analysis

compared the counts acquired on the Cell-Dyn for the EDTA blood and the ACD-A blood multiplied by the dilution factor.

IV. Resuspension and sample preparation of PRP for accurate counts

To determine the necessity of resuspension before platelet counts, three bovine PRP samples were analyzed using the Cell-Dyn 3700 immediately after preparation, and were then placed on the Ames Aliquot Mixer and counted every five minutes thereafter for a total of 20 minutes.

An additional evaluation was performed to determine if a small aliquot could be aspirated from the GPSTM II disposable to provide an accurate representation of the entire PRP fraction. Using a pipette, 0.6 mL of the PRP sample was extracted immediately after vigorous shaking, as described above, and counted on the Cell-Dyn 3700. The remaining PRP sample was placed on a rocker and allowed to mix for 15 minutes and then counted again in order to compare to the 0.6 mL extraction.

V. Platelet counts for PRP created by the GPSTM II

As a culmination of the validation procedures and platelet concentrator studies, 153 bovine PRP samples, using blood from approximately 8 different cows, were created using the GPSTM II concentration system. The volume of each PRP sample was recorded and each sample was counted for platelets using the Cell-Dyn 3700 three times. The corresponding baseline whole blood counts were also generated three times. Fold increase (PRP platelet concentration/baseline whole blood platelet concentration) and percent recovery (((PRP concentration x volume of PRP)/(baseline blood concentration x 60 cc)) x 100) were calculated. In order to compare the performance of the GPSTM II in concentrating human blood as compared to bovine blood, efficacy testing of the GPSTM II for concentrating human blood was also performed using three human samples.

VI. Statistical Analysis

Data is presented as mean \pm one standard deviation. Significant equivalence was determined either with a one-way analysis of variance (ANOVA) and a Student-Newman-Keuls test, or with a student t-test, using 95% confidence ($\alpha=0.05$).

RESULTS

I. Validation of platelet counts on a hematology analyzer

In this study, platelets in three PRP preparations were counted on the Cell-Dyn 3700 automated hematology analyzer and manually under a microscope at 400x magnification (n=21 for each preparation). There was an average difference of 1.1% between the manual count and the hematology analyzer counts (Table 1).

The statistical evaluation demonstrated that there was no significant difference found

PRP preparation	Manual (x10 ³ /μL)	Cell-Dyn 3700 (x10 ³ /μL)	Percent difference	T-test p values
1	2442 ± 164	2472 ± 47	1.2%	0.212
2	3192 ± 236	3232 ± 136	1.2%	0.256
3	2898 ± 209	2875 ± 71	0.79%	0.321
Averaged coefficient of variation	7.1%	2.9%	Average 1.1%	

Table 1-PRP platelet counts. Data presented as mean ± standard deviation (n=21 per sample).

between the manual and automated platelet counts (p>0.05). Furthermore, in this study, the average coefficient of variation for the Cell-Dyn counts (2.9%) was lower than the variation for the manual counts (7.1%), suggesting that the Cell-Dyn 3700 can be used to count platelets in PRP samples with greater reproducibility than manual counting.

II. Increase in linear range for the hematology analyzer

The manufacturer limit for the linear range for platelet counts of the Cell-Dyn 3700 is 2,000x10³ platelets/μL. In order to demonstrate linearity in ranges higher than the manufacturer limit, concentrated PRP samples were counted at full strength, then diluted 1:2 and 1:4 and counted again. In Figure 1, the averaged full strength platelet count is compared to the 1:2 dilution multiplied by 2 and the 1:4 dilution multiplied by 4. Statistical analysis indicated no statistical difference between the full strength and diluted samples multiplied by the corresponding dilution factor (p>0.05). In addition, the linear correlation value between the full strength and the diluted platelet counts was found to be R²=0.96 (data not shown). These results suggest that no dilution is necessary to count PRP samples with concentrations up to approximately 4,800x10³ platelets/μL.

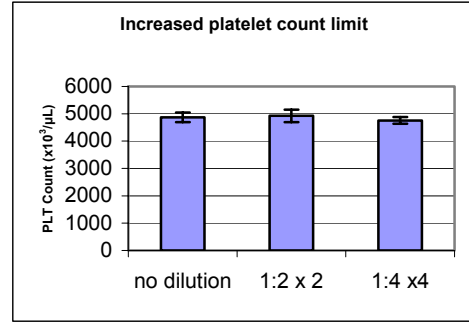


Figure 1-Platelet counts of PRP samples corrected for dilution ratios of 1X, 2X, and 4X, respectively. Data presented as average ± 1 stdev. Student-Newman-Keuls test indicates no statistical difference in counts (p>0.05).

III. Validation of platelet counts with citrate anticoagulated blood

The Cell-Dyn 3700 is manufacturer-validated only with blood anticoagulated with EDTA. Human blood was drawn into EDTA-coated vacutainer tubes (no dilution of whole blood) or was drawn into 7 mL uncoated vacutainer tubes with 0.8 mL of ACD-A added (dilution factor 1.11). Since PRPs could not be prepared using the same K₂ EDTA, only baseline blood samples were compared. Figure 2 summarizes the results for the platelet counts between EDTA and ACD-A anticoagulated human blood. The average platelet count for the EDTA blood for all three individuals was 220,000 platelets/μL ± 17,000 platelets/μL, while the average platelet count for the equivalent ACD-A blood multiplied by the dilution ratio was 214,000 platelets/μL ± 17,000 platelets/μL. These values were found to be statistically the same (p>0.05).

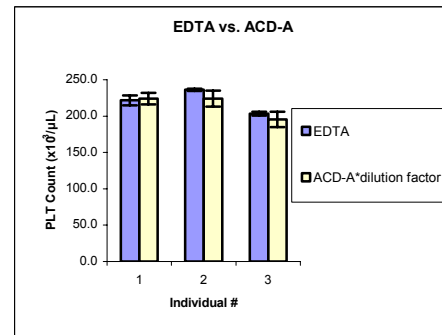


Figure 2- Platelet counts for blood of three human samples anticoagulated with EDTA and ACD-A anticoagulant. No significant difference between EDTA platelet average and ACD-A platelet average multiplied by the dilution factor of 1.11 (p>0.05). Data presented as average ± 1 stdev.

Therefore, the Cell-Dyn is capable of counting ACD-A anticoagulant blood accurately. If results are to be compared to other forms of anticoagulated blood, the dilution factor must be considered.

IV. Resuspension and sample preparation of PRP for accurate counts

Adequate resuspension of the PRP and correct sample preparation is required for accurate platelet counts. A time course of platelet counts while on a rocker to aid in resuspension is shown in Figure 3. In all three samples, the average platelet count was significantly less when the sample was measured immediately after preparation of the PRP than when the samples were resuspended on the rocker ($p < 0.05$). Following 5 minutes on the rocker, the platelet counts increased and remained constant throughout the 20 minute time course ($p > 0.05$). These results demonstrate the importance of agitation of the PRP sample for at least 5 minutes on a rocker before attempting to perform a platelet count.

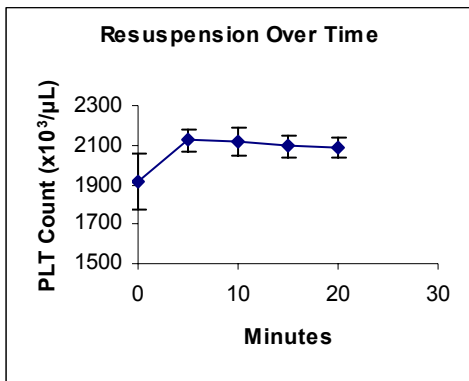


Figure 3-Time course of platelet resuspension while on a rocker. Three different individual samples were measured in triplicate at each time point. The time zero time point was significantly lower than all the other time points measured. Data presented as average \pm 1 stdev.

When a 0.6 mL sample was extracted from the PRP after shaking the disposable but before resuspension on the rocker and counted, the platelet count was significantly less than the same full sample resuspended (Figure 4). The average platelet count for the 0.6 ml samples was $1,958 \times 10^3$ platelets/ μ L \pm 140×10^3 platelets/ μ L as opposed to $2,093 \times 10^3$ platelets/ μ L \pm 56×10^3 platelets/ μ L for the full sample ($p = 0.007$).

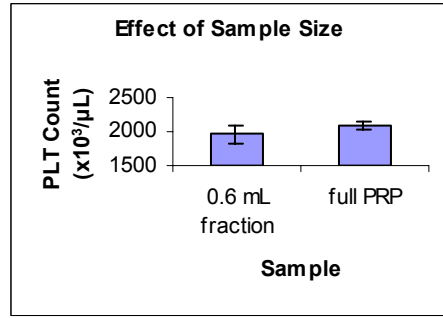


Figure 4-Comparison of platelet counts from 0.6 mL sample removed from the PRP sample for counting compared to the entire sample resuspended for 15 minutes and then counted. The 0.6 mL fraction counts were significantly less than the full resuspended sample ($p = 0.007$). Data presented as average \pm 1 stdev.

These data support the premise that an accurate platelet count cannot be guaranteed when a portion of the PRP is removed for counting. However, in another experiment, it was demonstrated that the samples could be divided in half and accurately counted after the entire PRP sample was allowed to resuspend on the rocker for 15 minutes (data not shown).

V. Platelet counts for PRP created by the GPS™ II

Both bovine and human PRPs were created with the GPS™ II, and fold increase in platelet concentration and percent recovery of whole blood platelets were calculated. With the bovine blood, the average baseline whole blood count was 328,000 platelets/ μ L \pm 69,000 platelets/ μ L. Following concentration with the GPS™ II, the samples were concentrated to an average 2,645,000 platelets/ μ L \pm 680,000 platelets/ μ L (Figure 5).

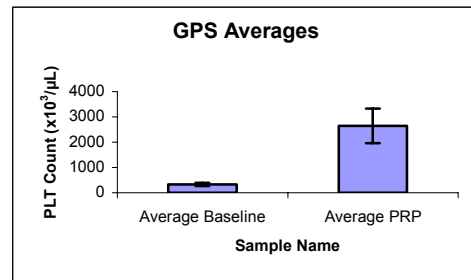


Figure 5-PRP platelet concentration for 153 samples concentrated using the GPS™ II. Data presented as average \pm 1 stdev.

The average fold increase for the 153 bovine PRP samples created with the GPS™ II was 8.06 ± 1.14 and the average percent recovery for the same samples was $75.7\% \pm 9.3\%$. Table 2 gives the results of the human platelet counts. The

average baseline platelet count for all three human samples was 194,000 platelets/ μL \pm 59,000 platelets/ μL and the PRP platelet count for the three samples was 1,845,000 platelets/ μL \pm 353,000 platelets/ μL .

	Baseline Average (x10 ³ / μL)	PRP Average (x10 ³ / μL)	Fold Increase	Percent Recovery
Sample 1 (5 cc)	148	1605	10.87	90.6%
Sample 2 (6 cc)	173	1680	9.71	97.1%
Sample 3 (6 cc)	261	2251	8.64	86.4%
Average	194 \pm 59	1845 \pm 353	9.7 \pm 1.1	91.2% \pm 5.5%

Table 2-Human platelet counts using the GPSTM II concentration system (each specimen counted in triplicate).

The average fold increase as a result was 9.7 \pm 1.1 and the average percent recovery was 91.2% \pm 5.5%. These data illustrate that the GPSTM II can concentrate both human and bovine blood effectively, and both can accurately be counted using the current sample preparation steps described in this study.

DISCUSSION

In order to determine the efficacy of platelet rich plasma (PRP), it is first necessary to accurately quantitate the platelet concentration. Only then can the number of platelets required for each surgical application be addressed. Hematology analyzers are routinely used to accurately and precisely count elements within blood. However, most systems are designed to operate in ranges found within whole blood. Therefore, counting platelets in concentrations found in PRP can generate errors or spurious results. There are mechanical limitations in both the hematology analyzers and the specimen preparation steps that can effect the accuracy of platelet counts. The purpose of this study was to validate one type of hematology analyzer for counting platelets in PRP and to define the specimen preparation methods needed to ensure accuracy. The validations performed here were designed for this particular counter, and validation for other hematology analyzers would need to be designed according to the device's specific operational parameters and limits.

Both bovine blood and human blood were used to generate PRPs. The platelets were counted on the Cell-Dyn 3700. Through manual counting techniques, it was apparent that the

GPSTM II adequately produced bovine PRP and that the fold increase from baseline blood was similar to that seen in human PRP preparations. Additionally, the Cell-Dyn 3700 is equipped with a veterinary package, allowing the system to be used for multiple species. For hematology analyzers to count platelets of any species, the machine must be able to discern red blood cells (RBC) from platelets according to size. Human RBCs have a mean cell volume of approximately 79-97 fL, while the platelets have a mean platelet volume of 5.6-10.4 fL (15). Bovine RBCs and platelets are similarly sized, with a mean cell volume of 40-60 fL and a mean platelet volume of around 7 fL (16). The size difference between the RBCs and platelets are large enough for the Cell-Dyn to discern between the two cell types of particles. This is not necessarily the case for other species. For instance, goats RBCs are only 23 fL while their platelets are 4.4-8.3 fL (15). In a preliminary study, we were unable to count goat platelets from a PRP using the Cell-Dyn 3700. Our counts were consistently falsely high, presumably because the system was counting both RBCs and platelets as platelets (Data not shown). It is assumed that for the purposes of this study, PRP preparations with either human or bovine blood were equivalent in platelet concentration and with the capacity of the Cell-Dyn 3700 to generate accurate counts.

The first validation step was to evaluate the Cell-Dyn 3700 platelet counts in PRPs as compared to manual counts. This technique was used by Veillon *et al.* in order to evaluate the accuracy of several different hematology analyzer systems by different manufacturers (17). In this study, the platelet counts of the PRPs were statistically equivalent to the manual counts. The Cell-Dyn was also capable of more precision, as evident by lower coefficient of variation than the manual counts. In addition to being able to count platelets in PRPs accurately, the Cell-Dyn 3700 is equipped to provide a warning flag if platelet clumps are detected. Counts with platelet flag clumping errors were discarded and new PRP samples were counted.

The Cell-Dyn 3700 was validated by the manufacturer to count platelets up to 2,000x10³ platelets/ μL , a level the GPSTM PRP preparation can exceed. It is important to understand the linear limit of the hematology analyzer to determine whether specimen dilution is necessary for that particular system. Concentrated PRPs were created and counted at full strength and at a 1:2 (50%) and 1:4 (25%) dilution. When the counts were plotted and a

linear regression analysis was performed, a $R^2=0.96$ correlation was found. Furthermore, as shown in Figure 1, when the diluted platelet counts were multiplied by the dilution factor, the values were found to be statistically equivalent to the undiluted samples. Taken together, these results suggest that the Cell-Dyn 3700 can be used to count PRP up to approximately $4,800 \times 10^3$ platelets/ μL without a dilution step.

The last system parameter validated was platelet counts with blood anticoagulated with ACD-A. Citrate-based anticoagulants in liquid form are often used in blood bags and in stored platelet populations because they can maintain platelet viability and metabolism better than EDTA (14;18). Most platelet concentration devices recommend the use of citrate anticoagulated blood with their systems. However, the Cell-Dyn's manufacturer only validated the system with blood anticoagulated with EDTA. This anticoagulant is coated onto the inside of vacutainer tubes and does not significantly dilute the blood. Our results demonstrated that the platelet counts in ACD-A anticoagulated blood, once multiplied by the dilution factor, were equivalent to counts for samples from the same donor anticoagulated with EDTA. Again, each anticoagulant used should be validated on the system being used for platelet counts. PRPs could not be compared between the two anticoagulants because K_2 EDTA tubes are not available in a large enough volume.

After determining that the counting system would function appropriately, focus was then placed on the requirements for sample preparation of the platelet concentrate. As demonstrated by the time course of the PRP on the rocker, at least five minutes of agitation ensures that the PRP is adequately mixed. When the platelets are first removed from the centrifuge and resuspended by 30 seconds of shaking, the platelets are not evenly distributed and can clump. This clumping does not result in full platelet activation, as demonstrated by low p-selectin expression on the PRP platelets (1). Without providing time for the platelet clumps to relax, low platelet counts and platelet error flags on hematology analyzers can occur.

A common practice used in the surgical arena is to collect a PRP for a patient, and then to aspirate off a small fraction for counts. The remaining PRP is given back to the patient for therapeutic use. As demonstrated in this study, sample fractionating cannot provide accurate platelet counts. Following PRP preparation, the

platelets are not evenly distributed and may be clumped. In this study, we demonstrated that a 0.6mL fraction gave a statistically significant lower platelet count than the same full sample allowed to rest on the shaker for 15 minutes. The smaller fraction of PRP before shaking on the rocker could have lower platelet counts either because platelets in the disposable may be slightly clumped or the platelets may not be evenly distributed throughout the entire PRP volume. Therefore, this study suggests that for accurate platelet counts to be achieved, the entire PRP sample must be removed and allowed to sit on the rocker at least 5 minutes before counting.

In addition, this study demonstrated that PRP samples should be mixed immediately prior to being counted. This study suggests that using manual modes in the hematology analyzers are more beneficial than automatic counting modes in order to prevent tubes of PRP to have time to settle before counting. The automatic counting mode on the hematology analyzer can allow the blood sample to settle for some time before counting occurs. Manual counting methods ensure that the blood sample is agitated up to the moment it is counted. The amount of platelet settling and resuspension may vary depending on the red blood cell (RBC) percentage in the PRP. PRP preparation systems can vary in the amount of platelets concentrated and the amount of RBCs that are removed, so each platelet concentrate system could require an individualized sample preparation and resuspension protocol.

Once the hematology analyzer and the PRP preparation device are validated, then accurate platelet counts can be achieved. As shown in Figure 5 and Table 2, the GPSTM II can provide an 8 fold increase in platelet concentration over baseline, with a greater than 70% platelet recovery. These data are consistent with a 10 patient clinical study that analyzed platelet counts and growth factor content in platelets collected with the GPSTM System (1).

The many issues that can preclude accurate platelet counts from a PRP using an automated machine have been demonstrated in this study. With several platelet concentration devices commercially available, comparison of the outputs of these devices is inevitable (19;20). However, without accurately validating the hematology analyzer and preparing the PRP samples appropriately for each system being compared, the results may be inaccurate and misleading.

PRPs have demonstrated numerous clinical benefits to patients (21-25). There are many devices on the market that concentrate platelets to differing levels. At this time, it is unclear the amount of platelets that is most efficient for each surgical application. Marx recommended at least $1,000 \times 10^3$ platelets/ μL are generally required (11). The GPSTM system can provide platelets up to 8 times baseline concentration (1), and the normal human platelet range is 200×10^3 platelets/ μL - 400×10^3 platelets/ μL . This implies that the GPSTM provides platelets in a range of $1,600 \times 10^3$ platelets/ μL - $3,200 \times 10^3$ platelets/ μL . In order to

further understand the amount of platelets required for each application, adequate platelet counting protocols must be established for platelets in the ranges discussed above. This study addresses the issues in ensuring the hematology analyzer used can accurately count the platelets in the PRPs, and that the PRPs are prepared in such a way as to provide accurate counts. Only after these steps have been followed, can comparison between devices be made.

ACKNOWLEDGEMENT

All work was funded by Biomet, Inc.

References

- (1) Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004 Nov;114(6):1502-8.
- (2) Weibrich G, Kleis WK, Buch R, Hitzler WE, Hafner G. The Harvest Smart PRP system versus the Friadent-Schutze platelet-rich plasma kit. *Clin Oral Implants Res* 2003 Apr;14(2):233-9.
- (3) Kevy SV, Jacobson MS, Blasetti L, Fagnant A. Preparation of growth factor enriched autologous platelet gel. *27th Society for Biomaterials* 24, 262. 2001.
Ref Type: Abstract
- (4) Slater M, Patava J, Kingham K, Mason RS. Involvement of platelets in stimulating osteogenic activity. *J Orthop Res* 1995 Sep;13(5):655-63.
- (5) Spencer ME, Tokunaga A, Hunt TK. Insulin-like growth factor binding protein-3 is present in the alpha-granules of platelets. *Endocrinology* 1993;132(3):996-1001.
- (6) Mishra A, Pavelko T. Treatment of severe lateral epicondylitis with platelet rich plasma. *American Academy of Orthopedic Surgeons* . 2005.
Ref Type: Abstract
- (7) Lowery GL, Kulkarni S, Pennisi AE. Use of autologous growth factors in lumbar spinal fusion. *Bone Supplement* 1999 Aug;25 No.2:47S-50S.
- (8) Aminiam B, Shams M, Soveyd M, Omrani GhR. Topical autologous platelet-derived growth factors in the treatment of chronic diabetic ulcers. *Archives of Iranian Medicine* 2000.
- (9) Bhanot S, Alex JC. Current Applications of Platelet Gels in Facial Plastic Surgery. *Facial Plastic Surgery* 2002 Nov;18(1).
- (10) Garg AK. The use of platelet-rich plasma to enhance the success of bone grafts around dental implants. *Dent Implantol Update* 2000 Mar;11(3):17-21.
- (11) Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 2004 Apr;62(4):489-96.
- (12) Wertz RK, Koepke JA. A critical analysis of platelet counting methods. *Am J Clin Pathol* 1977 Jul;68(1 Suppl):195-201.
- (13) Marx RE. Platelet concentrate: a strategy for accelerating and improving bone regeneration. In: Davies JE, editor. *Toronto: University of Toronto*; 2000. p. 447-53.
- (14) Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent* 2001;10(4):225-8.
- (15) Lewis JH. *Comparative Hemostasis in Vertebrates*. New York and London: Plenum Press; 1996.
- (16) Jain NC. *Essentials of Veterinary Hematology*. Lippincott, Williams, and Wilkons; 1993.
- (17) Veillon DM, Curry S, Tubbs K, Kaltenbach J, Cotelingam JD. Decision dilemma: How we chose a new hematology analyzer. *Laboratory Hematology* 2000;6:151-6.
- (18) Landesberg R, Roy M, Glickman RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. *J Oral Maxillofac Surg* 2000 Mar;58(3):297-300.
- (19) Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *JECT* 2004;36:28-35.
- (20) Waters JH, Roberts KC. Database review of possible factors influencing point-of-care platelet gel manufacture. *J Extra Corpor Technol* 2004 Sep;36(3):250-4.
- (21) Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 2001 Mar;24(3):483-8.
- (22) Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998 Jun;85(6):638-46.
- (23) Mooar PA, Gardner MJ, Klepchick PR, Sherk HH. The efficacy of autologous platelet gel in total knee arthroplasty: an analysis of range of motion, hemoglobin, and narcotic requirements. *American Academy of Orthopaedic Surgeons*; 2000 p. PE148.
- (24) Welsh WJ. Autologous platelet gel-clinical function and usage in plastic surgery. *Cosmetic Dermatology* 2000;13-8.
- (25) Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg* 1997;1294-9.

