Platelet yield and yield consistency for six single-spin methods of platelet rich plasma preparation

Theodore E. Harrison, Jannice Bowler, Todd N. Levins, An-Lin Cheng & K. Dean Reeves

To cite this article: Theodore E. Harrison, Jannice Bowler, Todd N. Levins, An-Lin Cheng & K. Dean Reeves (2019): Platelet yield and yield consistency for six single-spin methods of platelet rich plasma preparation, Platelets, DOI: 10.1080/09537104.2019.1663808

To link to this article: https://doi.org/10.1080/09537104.2019.1663808

Published online: 09 Sep 2019.
Platelet yield and yield consistency for six single-spin methods of platelet rich plasma preparation

Theodore E. Harrison¹, Jannice Bowler², Todd N. Levins³, An-Lin Cheng⁴, & K. Dean Reeves⁵

¹Rejuvacare PC Regenerative Medicine, Victoria, BC, Canada, ²Family Physician, Victoria, BC, Canada, ³Rise Health, Victoria, BC, Canada, ⁴Biostatistician, Department of Biomedical and Health Informatics, University of Missouri-Kansas City, School of Medicine, Kansas City, Missouri, USA, and ⁵Private Practice Physical Medicine and Rehabilitation, Roeland Park, Kansas, USA

Abstract

Single-spin methods of preparation of platelet-rich plasma are used widely in private practice, yet they have not been extensively studied and compared. The use of platelet-rich plasma (PRP) by the private practitioner can be facilitated by efficient and predictable PRP preparation. The primary purpose of this study was to study common methods of single-spin PRP preparation to determine their efficiency and variability. Six single-spin methods of PRP production from whole blood were analyzed. The primary measures were mean yield and standard deviation as the quotient of total platelet count in PRP produced divided by total platelet count in whole blood utilized. Each sample was analyzed in triplicate and the results were averaged. Secondary measures included red blood cell count (RBC) and white blood cell count (WBC), concentration ratio, and variable cost per million platelets produced. Sixty-four volunteers provided samples from 30 June 2017 to 30 September 2018. Seventeen to twenty six samples were utilized to assess each method. Yields for the six preparation methods (PMs) varied from 53(±18)% to 72(±13)%. Differences were observed for WBC count (1.8 to 14), Hematocrit (0.8 to 32), platelet concentration (568 to 1062), and variable cost per billion platelets produced ($1.55 to $44.31). All six methods evaluated provided a platelet yield of more than 50%, although two methods were less efficient than the others. Two methods were able to produce leukocyte-poor PRP. Variability was moderate across all methods, suggesting that estimation of platelet yield should be feasible from a baseline platelet count for all methods.

Introduction

In 2001 Marx [1] defined platelet-rich plasma as plasma containing one million platelets per microliter. He further asserted that adequate PRP could not be made using a single-spin method and that a double –spin method using first a soft spin to remove red blood cells (RBC) and then a hard spin to concentrate the platelets (PLT) was required. Liu [2] in 2002 and then Vasquez [3] in 2004 went on to show that the concentrations of growth factors released from platelets in PRP were the critical factors in driving new tissue growth, and thus that the number of platelets in a PRP preparation is the most important element of its usefulness.

In the ensuing years the use of PRP has spread from dentistry rapidly through the surgical and other specialties, especially musculoskeletal [4]. New devices for PRP preparation have been invented and commercialized. Further investigation of the properties and physiology of PRP have brought a better understanding of how it works. With the advent of stem cell therapies PRP has been recognized as a valuable adjunct [5].

There is Level II evidence of efficacy for the use of PRP in knee osteoarthritis [6] and lateral epicondylitis [7]. A dose response curve has not been established for use of PRP for these or any other condition. However, tissue culture studies have shown dose-dependent proliferation of cells treated with PRP and we may expect clinical studies to follow.

When a dose response curve for platelet dosage is established for a given condition, there are four practical issues that remain for the treating physician. One is the need to estimate the delivered dose of platelets from the preparation method. This is difficult because of the inconvenience and expense of transporting samples of every patient’s PRP to a local laboratory for analysis and the impracticality of maintaining a hematology analyzer in most offices. The second is the time involved in PRP preparation, favoring single spin methods. The third is the need to be aware of other characteristics of PRP produced, such as RBC and WBC counts and platelet concentration, and the fourth is the cost involved in PRP preparation.

A number of studies [8,9–13] have been done comparing different methods of preparing PRP, but most focus has been on double-spin methods, which take significantly longer to perform than single-spin methods. The purpose of this study was to investigate common single-spin methods of PRP preparation to determine (a) comparative yields and consistency of yield as a preliminary indication of the practicality of each method in reaching a target dose, and (b) secondary characteristics (such as RBC, WBC counts and platelet concentration) of the PRP.

Keywords

Platelet rich plasma, platelets

Correspondence: Theodore E. Harrison, Rejuvacare PC Regenerative Medicine, Victoria, British Columbia, Canada. E-mail: teh@rejuvacare.org
produced by each method, as debate continues about the optimum PRP characteristics for any given indication [14,15].

This study is the first phase of a two-part study in which we propose to attempt to overcome the difficulty of determining platelet dosage by: (1) testing several PRP preparation methods (PM) to determine their yields and consistency, and (2) using this information to predict PRP platelet yields from a CBC platelet count. Here we report our phase one results, in which we tested six different single-spin methods of PRP preparation for yield and consistency. This study was reviewed and approved by the ICMS Institutional Review Board: ICMS Approval Number: ICMS-2017-003.

**Materials and Methods**

**Preparing for the Study**

This study involves several techniques that have not been published in detail. Sometimes the manufacturer’s instructions were not sufficient to determine optimal parameters. Therefore we had to do preliminary testing in these cases in order to establish the best parameters for producing PRP. This involved testing different centrifuge speeds and times, and comparing results until we found the best combination. In addition we could not find any recommendations as to exactly how much of the buffy coat to include in the PRP for any of the techniques that required buffy coat aspiration, so we conducted several trials to determine the best volume for each technique.

**Inclusion/Exclusion Criteria**

Subjects were recruited from friends, family, colleagues and patients seen at the two study sites. Subject to time constraints, all subjects seen at the study sites were offered screening. Volunteers with cancer, blood dyscrasias, known platelet problems, platelet counts outside the reference range and who were taking drugs or hormones known to effect platelet production and function were excluded. Eligible patients that were interested in participating signed informed consent.

**Centrifuge Speeds and Calculation of G-forces**

All relative centrifugal forces (RCF, g) were calculated from the midpoint of the column of blood in the centrifuged tube (Rmid, in cm). RCF is a nonlinear function of the radius of the centrifuge rotor and the speed (rpm, revolutions per minute) of the centrifuge using the following formula:

\[ RCF = 1.12 \times \text{Radius} \times (\text{rpm}/1000)^2 \]

In the centrifugation of whole blood the components separate into layers: red blood cells, white blood cells, platelets and plasma. For single-spin PRP preparation the target layer is the platelet layer, which settles in the middle of the column of blood components, the exact level being determined by the subject’s hematocrit. Since the preparations were made using several different centrifuges and since we wanted others to be able to reproduce these results on a variety of centrifuges, we opted to use Rmid, the radius from the center of the centrifuge rotor to the middle of the column of blood, as the radius from which to calculate the g-force. We felt that this would result in more reproducible results from lab to lab (or clinic) than using the usual Rmax (the distance from the center of the rotor to the bottom of the column of blood), which targets the bottom of the column of fluid.

**Preparation Methods**

Each participant signed informed consent and gave blood samples that were used to prepare PRP in up to six different ways. In some of the commercial kits we found that the optimal yields required somewhat different methods than those recommended by the manufacturer.

The detailed methods were as follows:

- **ACD (yellow-top, YTT) tube method** (after Peterson and Reeves [16])
  - 8.5cc of whole blood was collected in a standard ACD-containing yellow-top tube (BD Vacutainer ACD, catalog #364606; Becton-Dickinson, Franklin Lakes, NJ, USA).
  - The tube was placed in a test tube centrifuge and spun at 1000g for 10 minutes.
  - The supernatant platelet-poor plasma (PPP) was drawn off from the top of the plasma layer leaving 1-2cc of PRP.
  - 1-2cc of PRP was withdrawn from just above top of the RBC layer.

- **Double-syringe (Arthrex ACP®) Method**
  - 15cc of whole blood was drawn into an ACP double syringe (Arthrex ACP Double-Syringe System; Arthrex Inc., Naples, Florida, USA).
  - The syringe was immediately placed in an Arthrex lab centrifuge and spun at 1500rpm (300g) for five minutes.
  - The entire plasma layer was withdrawn using the internal syringe.
  - The resultant PR was anticoagulated with ACD-A in a 1:10 ratio for purposes of the study.

- **Single 20cc Syringe Method**
  - 1.5cc of Sodium citrate (NaCi) 40mg/ml was drawn up into a standard plastic 20cc syringe.
  - 15cc of whole blood was then drawn into the syringe and mixed well.
  - The plunger shaft and flanges of the syringe were cut off; the end was capped; and the syringe placed into a lab centrifuge and spun at 1000 g for 10 minutes.
  - All but the bottom 4-4.4cc of the plasma layer was removed.
  - The rest of the plasma layer plus 0.6cc of the red layer was then removed and mixed thoroughly. The 0.6cc of the red layer was included because on aliquot testing we found that this was where most of the platelets were located.

- **Narrow-neck Tube Method (YCellBio)**
  - 1.5cc of ACD-A was drawn up into a 20cc plastic syringe.
  - 15cc of whole blood was then drawn into the syringe and mixed well.
  - 15cc of the anticoagulated blood was transferred to a narrow-neck tube (YCell Bio Blood Separation Kit; YCellBio, Seoul, South Korea).
  - The narrow-neck tube was placed in a test tube centrifuge and spun at 1000 g for 10 minutes.
  - The bottom 4cc of plasma plus the top 0.12cc of the red layer were withdrawn and mixed well. The 0.12cc of the red layer was included because on aliquot testing we found that this was where most of the platelets were located.

- **Gel-separator Tube Method**
  - Gel-separator tubes were purchased from Regen Lab (RegenKit A-PRP; Regen Lab SA (Switzerland), En Budron B2 CH-1052), Eclipse Esthetics (Eclipse PRP kit; Eclipse Esthetics LLC, The Colony, Texas, USA) and Suzhou Runfeng Network Technologies, (PRP Tube ACD+Gel; Suzhou Runfeng Network Technologies, Chengyang, Qingdao, China).
–10–12cc of whole blood was drawn into a gel-separator tube containing 1cc ACD-A and 1–2cc of a gel with a slightly higher density than platelets.
–The tube was placed into a test tube centrifuge and spun at 900–975 g for 10 minutes.
–From the top of the sample all but about 3cc of the plasma layer were removed.
–The remaining plasma was swirled in the tube to re-suspend the platelets.
–The PRP was withdrawn from the tube.

**Machine (Arthrex Angel®) Method**

Two or three 60cc syringes were prepared with 5cc of ACD-A each.

–90–180cc of whole blood was drawn into the syringes and mixed well.
–The anticoagulated blood was placed into the Angel® machine (Angel® Concentrated Platelet Rich Plasma System; Arthrex Inc., Naples, Florida, USA) and processed for 23 minutes with the setting set for a hematocrit of 4%.
–The PRP obtained was diluted 5:1 with PPP because the undiluted PRP was too concentrated to be analyzed by the hematology analyzer.

**Measures**

Each participant had a simultaneous complete blood count (CBC) with platelet count (PLT) (MSLAB-7 Full-Auto Hematology Analyzer, Guangzhou Medsinglong Medical Equipment Co. Ltd. Guangzhou, China). Each CBC and PRP sample was analyzed in triplicate and the results averaged. Total whole blood (WB) PLT and PRP PLT were calculated for each sample and the yield (PRP PLT/WB PLT) derived. Mean yield and standard deviation (SD) were the primary measures and were calculated for each PM. WBC concentration and RBC concentration were also measured for each PRP sample.

**Analysis**

Sample sizes for each PM were determined for ±10% Confidence Interval (CI) and 95% Confidence Level (CL) using the formula n = (Z^2 × SD^2)/(MoE)^2, where n is the required sample size; Z is the z-score for 95% confidence level; SD is the standard deviation; and MoE is the desired margin of error (CI). After testing five samples for each method we calculated a working mean and SD. As more data points were added to each PM data set the mean and SD were recalculated and the required sample size updated.

Baseline between-group data were analyzed for significant between-group differences by t-tests for nominal variables (Age, whole blood hematocrit, WBC, and platelet count) and Pearson chi square tests for categorical variables. (Gender) Analysis of the effect of baseline variables on yield was assessed using linear mixed model analyses, taking into account the repeated measurements resultant from participants providing blood samples that were used for several different preparation methods. If any baseline variable was found to have a significant effect on yield, it was included in statistical analysis of the effect of method on percentage yield, which also utilized a linear mixed model analysis. Data analysis was performed using SAS version 9.4 (SAS Institute, Cary, N.C.) with two-tailed tests and an alpha level of .05.

Platelet production per dollar was determined by dividing the mean total platelets produced per preparation by the sum of supply costs and office overhead costs. Supply costs included both the cost of required production kits and variable single use components such as syringes, needles, tubes, and drawing needles that were not included in the kit cost. Office overhead costs were determined by multiplying the average overhead cost of physicians by the estimated time of production. An office overhead cost estimate of $137 per hour was obtained by multiplying the mean annual physician salary by the average overhead cost as a percentage of salary and dividing by a mean number of office hours per year. The fixed cost components (centrifuge and International Organization Standardization (ISO) class V hood) were not included in the cost determination.

**Results**

Over a period from June 2017 through August 2018 64 volunteers were recruited from among friends, family, colleagues and patients. Eight samples were excluded from analysis, either due to system testing (2), idiopathic thrombocytopenic purpura (1), incomplete documentation (1), PRP clotting (1), sampling error (1), or other laboratory errors (2). Fifty six participants provided blood used for 141 analyses across the 6 methods tested. Each method data set met or exceeded the target confidence interval and confidence level estimated from the first five analyses for each method.

Baseline comparison of gender, age, and whole blood characteristics revealed a significant between group difference for gender. (p = .014; Table I) However, the only baseline variable that significantly affected platelet yield was the whole blood hematocrit (p = .017; Table I), with lower hematocrit levels associated with a higher platelet yield. The effect of method on yield, accounting for whole blood hematocrit, was significant, (p < .0001), although all yields exceeded 50%, ranging from 53 to 72%.

Using the volumes we tested the machine method had the highest platelet concentration and was the only PM that achieved the PRP definition of 1M Platelets/µL (“standard” PRP). However, it should be kept in mind that the platelet concentration is dependent on the amount of plasma that is withdrawn along with the platelets and that this is a controllable variable in all of the PMs except the double-syringe (DS) method. Thus all the methods except the DS method had the potential to create standard PRP. However, the DS method PRP had the lowest WBC counts and was the only method other than the gel-separator (GT) method to produce leukocyte-poor PRP. DS and GT methods also had the lowest hematocrit PRP.

The machine method was the most consistent, with only 11% SD, but it tied for lowest yield. The double-syringe method had the highest yield and with good consistency.

While costs will vary depending on location, Table II lists the components of cost analysis and platelet production per dollar for each method. All costs are absolute costs and do not take into account the amount of PRP produced by each method, although in all cases except the Machine method the amount of PRP was roughly the same. The machine method used about eight times as much blood and produced about five times as much PRP (but at a higher PLT concentration) as the other methods. The single-syringe and yellow-top tube methods are the least expensive.

**Discussion**

This study found that although there were differences in yield and consistency among six different single-spin methods of PRP preparation, many of the methods were able to produce clinically useful PRP. In addition the WBC concentration and RBC concentration also varied significantly but some methods were able to produce leukocyte-poor PRP. Despite the variability, in some cases the SD of the yield was relatively low and therefore it
Table I. Comparison of platelet-rich plasma preparation methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Value (SD)</th>
<th>Gender M/ F</th>
<th>Age in years</th>
<th>WB Hct Pct.</th>
<th>WB WBC 1000s/ μL</th>
<th>WB Plt 1000s/ μL</th>
<th>WB mL</th>
<th>WB TotPlt 1000s/ μL</th>
<th>PRP Hct Pct</th>
<th>PRP WBC 1000s/μL</th>
<th>PRP Plt 1000s/μL</th>
<th>PRP mL</th>
<th>PRP TotPlt 1000s/μL</th>
<th>Yield2 in pct</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA (17)</td>
<td>Value: 12-May</td>
<td>55</td>
<td>43</td>
<td>6.2</td>
<td>253</td>
<td>128</td>
<td>32823</td>
<td>2.3</td>
<td>11.2</td>
<td>1070</td>
<td>17.4</td>
<td>17725</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>14.6</td>
<td>2.8</td>
<td>1.2</td>
<td>52</td>
<td>30.5</td>
<td>10693</td>
<td>1.2</td>
<td>7</td>
<td>552</td>
<td>4.8</td>
<td>7399</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>DS (21)</td>
<td>Value: Jun-15</td>
<td>55</td>
<td>40</td>
<td>7.9</td>
<td>290</td>
<td>15</td>
<td>4345</td>
<td>0.8</td>
<td>1.9</td>
<td>368</td>
<td>5.6</td>
<td>3156</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>15.5</td>
<td>4.4</td>
<td>2.2</td>
<td>87</td>
<td>0</td>
<td>1308</td>
<td>2</td>
<td>1.6</td>
<td>203</td>
<td>0.9</td>
<td>1168</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>SS (26)</td>
<td>Value: May-21</td>
<td>55</td>
<td>41</td>
<td>7.4</td>
<td>282</td>
<td>14</td>
<td>3843</td>
<td>2.4</td>
<td>11.4</td>
<td>637</td>
<td>4.1</td>
<td>2533</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>13.5</td>
<td>4</td>
<td>1.9</td>
<td>66</td>
<td>0.1</td>
<td>905</td>
<td>1.4</td>
<td>4</td>
<td>269</td>
<td>0.4</td>
<td>943</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>NN (28)</td>
<td>Value: Sep-19</td>
<td>55</td>
<td>40</td>
<td>7.5</td>
<td>280</td>
<td>15</td>
<td>4221</td>
<td>10.4</td>
<td>14.9</td>
<td>719</td>
<td>4</td>
<td>2874</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>13.8</td>
<td>4.8</td>
<td>2.1</td>
<td>67</td>
<td>0.5</td>
<td>983</td>
<td>1.4</td>
<td>4.7</td>
<td>266</td>
<td>0.3</td>
<td>1042</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>GT (26)</td>
<td>Value: Jul-19</td>
<td>53</td>
<td>42</td>
<td>6.9</td>
<td>280</td>
<td>11</td>
<td>3043</td>
<td>1.4</td>
<td>5.3</td>
<td>882</td>
<td>2.7</td>
<td>1933</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>11.2</td>
<td>4.1</td>
<td>2</td>
<td>57</td>
<td>1.8</td>
<td>647</td>
<td>2.6</td>
<td>4</td>
<td>549</td>
<td>1.2</td>
<td>715</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>YT (25)</td>
<td>Value: Sep-16</td>
<td>54</td>
<td>41</td>
<td>7.2</td>
<td>290</td>
<td>8</td>
<td>2421</td>
<td>32</td>
<td>14</td>
<td>616</td>
<td>2.1</td>
<td>1280</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: NA</td>
<td>15.3</td>
<td>5.2</td>
<td>2.2</td>
<td>82</td>
<td>0.5</td>
<td>678</td>
<td>10.9</td>
<td>5.1</td>
<td>248</td>
<td>0.5</td>
<td>580</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

1. METHOD = preparation method, SD = standard deviation, n = sample size, WB = whole blood, PRP = platelet-rich plasma, WBC = mean white blood cell count in thousands per microliter, Hct = mean hematocrit, Plt = mean platelet count in thousands/microliter, TotPlt = total number of platelets, MA = Arthrex Angel, DS = double-syringe, SS = single syringe, NN = narrow-neck tube, GT = gel separator tube, YT = ACD yellow-top tube.

2. The p value that represents the effect of method on yield, while accounting for WBHct in the model, was <.001. The sample size estimated to achieve 90% power level for determination of yield was met for each method varying from 94 to 94% (Confidence intervals of 6% MA and DS, 7% for SS, NN and YT, and 8% for GT).
may be possible to estimate PLT concentration in PRP made by these methods just from the PLT concentration in whole blood. This will be the subject of phase two of this study.

The platelet dosage of most PRP used clinically is unknown. Similarly, the “optimal” PRP dosage for most clinical applications is also unknown, even for the most commonly used applications [17]. Nevertheless, it seems clear from tissue culture research [18,19,21], that there can be both inadequate and excessive dosages. It has been shown that the most important active ingredient in PRP is the platelets. The tissue culture studies by Giusti, Jo and Wang mentioned above clearly show dose-response effects. There is still debate, however, as to whether the curve is linear, sigmoid or a bell curve. Further research is needed to clarify this issue and to determine in vivo dose-response. Whilst it is logical to conclude that platelets act in a dose-dependent manner and should be dosed according to the disease and patient, this is probably a major reason why the results of clinical studies with PRP have been so variable. Though beyond the scope of our study we recognize that platelets are biological entities and clinical response may be dependent on more than the absolute number of platelets delivered. Different PMs may yield different cytokine/growth factor profiles and this may also be a significant factor in the reported variability of efficacy of PRP treatment. Similarly, the activation (or not) of PRP with thrombin, calcium, shear, UV or other methods may contribute significantly to differences in efficacy.

Clinical dose-response studies of PRP have been slow in coming. We found only one in the literature and it was inconclusive [22]. One reason is the myriad ways to prepare PRP and the lack of an easy dose measurement method. Previous studies have shown that the platelet count remains stable over a period of five days or more [23]. Therefore, theoretically, knowing only the CBC platelet count and the platelet yield of a specific PRP preparation method, clinicians might be able to estimate the platelet dosage with reasonable accuracy.

In order to have a more useful measurement we chose to analyze the yields of the different preparation methods, i.e. the percentage of platelets in the whole blood that were recovered in the PRP. This has the additional benefits of allowing us to compare the efficiency and consistency (Standard deviation (SD) of each method).

The ideal preparation method would be both highly efficient and very consistent. If efficiency (yield) and consistency are known for a particular PM then it is easy for the clinician to calculate the amount of WB required to produce a specific dose of platelets. For example, if a dose of 6B platelets is needed from a patient with a WB platelet count of 250k/μL and the yield of the PM is 60% then the amount of WB necessary to produce that dose is

\[
WBVOL = \text{DOSE}/(\text{PLT} \times Y) = 40 \text{ mL}
\]

At present there is a chicken-and-egg problem with respect to the investigation of dose-response curves for PRP administration. Such studies are difficult because many potential investigators do not have a bedside hematology analyzer to determine platelet dosages. The degree of accuracy from estimating dosage from a CBC may not be enough for establishing a dose-response curve, but since the therapeutic window of PRP efficacy seems to be quite wide (judging from tissue culture reports) it may be adequate for clinical use.

A weakness of this study is that the gel-separator tubes were obtained from multiple sources. This may have led to a higher SD than if they were all obtained from a single source. Comparative quantitative studies of platelet concentrate production have real life limitations due to the cost to obtain and maintain a calibrated automated hematology analyzer. An alternative would be to count cells and platelets using a simple microscope with phase contrast capabilities.

Our study does not address qualitative differences between platelet concentrates produced. In order to assess qualitative differences, measurement of levels of cytokines and other proteins associated with platelet activity such as TGFβ, IGF1, TIMP-1, MMP-3, IL-6, and platelet factor 4, might be useful. Such qualitative analyses are limited by cost, such as for either pre-prepared arrays for cytokine measurement or ELISA kits. In vitro measurements will have limited clinical applicability, however, and real-life qualitative impact would require measurement of changes in vivo, such as changes in synovial fluid levels or measurement by microdialysis.

Phase one of this study demonstrated the yields and consistencies of six different single-spin PRP preparation methods. A clinician’s choice may be dependent on the setting in which the PM is used and the applications for which the PRP is used. Each method has advantages and disadvantages.

Acknowledgements

Many thanks to Ian Hampton for his review of the statistical methods.

Declaration of Interest Statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal

---

**Table II. Cost analysis comparison.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Supply cost including kits</th>
<th>Preparation time estimate</th>
<th>Overhead cost @ $137/hour</th>
<th>Total cost per preparation</th>
<th>Total number of platelets produced per preparation</th>
<th>Platelets produced per U.S. dollar</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>$234</td>
<td>40 min</td>
<td>$91.30</td>
<td>$325.30</td>
<td>17,725,000</td>
<td>54,488</td>
</tr>
<tr>
<td>DS</td>
<td>$113</td>
<td>10 min</td>
<td>$22.80</td>
<td>$135.80</td>
<td>3,156,000</td>
<td>52,012</td>
</tr>
<tr>
<td>SS</td>
<td>$3</td>
<td>20 min</td>
<td>$45.70</td>
<td>$48.70</td>
<td>2,533,000</td>
<td>29,844</td>
</tr>
<tr>
<td>NN</td>
<td>$62</td>
<td>15 min</td>
<td>$34.30</td>
<td>$96.30</td>
<td>2,874,000</td>
<td>15 min</td>
</tr>
<tr>
<td>GT</td>
<td>$102</td>
<td>15 min</td>
<td>$34.30</td>
<td>$156.30</td>
<td>1,933,000</td>
<td>14,182</td>
</tr>
<tr>
<td>YT</td>
<td>$2</td>
<td>15 min</td>
<td>$34.30</td>
<td>$36.30</td>
<td>1,280,000</td>
<td>35,261</td>
</tr>
</tbody>
</table>

1 Method = preparation method, Prep time = total preparation time, MA = Arthrex Angel, DS = double-syringe, SS = single syringe, NN = narrow-neck tube, GT = gel separator tube, YT = ACD yellow-top tube.

2 From 2019 https://www.medscape.com/slideshow/2019-compensation-overview-6011286#2 last accessed June 28, 2019. The average overhead cost is estimated as 47% of practice revenue per year, or $277,600, and per week (divided by 52) would be $5,338. Hours per week across the country combining is 38.9 for an overhead cost per hour of $5338/38.9 hours = $137/hour. The fixed cost components (centrifuge and International Organization Standardization (ISO) class V hood) were not included in the cost determination.
or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Funding

No financial or material support was received by any coauthor for the performance of this study.

References