Implementation of buffy coat platelet component production: comparison to platelet-rich plasma platelet production

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BACKGROUND: Buffy coat (BC) production of platelets (PLTs) has been successfully used in Europe for more than two decades. Currently, Canadian Blood Services is implementing the BC method. This article summarizes results of the validation testing performed to qualify the process of PLT production from whole blood and compares the quality of PLTs produced in routine production by either the PLT-rich plasma method (PRP-PCs) or the BC method (BC-PCs).

STUDY DESIGN AND METHODS: Validation data included variables used for routine quality control (QC; pH, PLT count, volume, sterility, residual white blood cell count) as well as nonroutine testing of PLTs for PLT activation, metabolic changes during storage, and PLT responsiveness to hypotonic shock and the extent of shape change induced by adenosine 5′-diphosphate. BC-PCs were tested on Days 1 and 6. QC of production runs included the same routine tests performed on Day 6.

RESULTS: PLTs produced by the BC method during validation and pilot implementation met all Canadian Standards Association standards with respect to yield, volume, pH, and leukoreduction. Additional validation testing indicated a moderate level of PLT storage lesion development. In comparison to PRP-PCs, in vitro variables of BC-PCs, either pH in this study, or other markers compared to the literature were better, suggesting that BC-PCs have less evidence of production-related damage and improved PLT quality during storage.

CONCLUSIONS: PLT concentrates produced from whole blood by the BC method after an overnight hold have laboratory variables suggestive of a higher quality than those concentrates produced by the PRP method.

Canadian Blood Services undertook to change its method of component production from whole blood from the North American standard platelet-rich plasma (PRP) method to the buffy coat (BC) method of platelet (PLT) production. Developed by investigators in the Netherlands and Sweden in the mid-1970s, the BC production method reverses the sequence of centrifugation steps compared to PRP. A hard spin is used initially to separate whole blood into three components: plasma, red blood cells (RBCs), and a BC layer. Using semiautomated extraction, the most common configuration uses a so-called top-and-bottom collection set in which plasma and RBCs are transferred to storage containers and the BC is left in the donation bag. This BC contains PLTs, white blood cells (WBCs), plasma, and some RBCs. ABO-matched BCs are pooled together with 1 plasma unit from one of the donors or 1 unit of PLT additive solution using a sterile docking device. The pooled BC is then given a soft spin, and the PRP is extracted with or without leukofiltration to produce a pooled PLT concentrate. Although both methods produce a PLT product, the products may have somewhat different in vitro characteristics. There is noticeable quality improvement in laboratory markers of BC-produced PLTs.

ABBREVIATIONS: BC(s) = buffy coat(s); BC-PC(s) = platelets prepared by the buffy coat method; HSR = hypotonic shock response; PRP = platelet-rich plasma; PRP-PC(s) = platelets prepared by the platelet-rich plasma method.

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(BC-PCs) relative to PRP-produced PLTs (PRP-PCs). This is characterized by a higher proportion of discoid PLTs, lower CD62 expression, and better hypotonic shock response (HSR) at the end of storage. Metcalfe and coworkers indicated that PLTs produced by the PRP method have a higher level of the activation markers CD62 and annexin V 1 hour after the end of the production process. BC-PCs are leukoreduced well below acceptance level, reducing risk for nonhemolytic transfusion reactions or alloimmunization.

As part of the process to validate the BC manufacturing method in the Canadian Blood Services’ environment, comparisons were made of PLT products against the existing Canadian Standards Association standards and an additional panel of in vitro tests was performed to assess PLT quality. This information was compared to the quality assessment of PRP-PCs produced in the routine manufacturing environment, as well as to subsequent quality control (QC) data obtained after conversion of the manufacturing process to BCs. The choice of validation tests performed at Canadian Blood Services Network Centre for Applied Development (NetCAD) was influenced by extensive experience in PLT evaluation and available literature on correlation of in vitro tests to PLT recovery.

MATERIALS AND METHODS

Collection and PLT production

The validation exercise was conducted at NetCAD, Canadian Blood Services’ development laboratory. Whole blood was collected from healthy donors in accordance with current Canadian Blood Services standard operating procedures. All donors gave written informed consent for participation in the study. Units were collected into top-and-bottom blood collection systems manufactured either by MacoPharma (Leukoflex LCR5 quadruple top-and-bottom blood pack unit, citrate-phosphate-dextrose [CPD]/saline-adenine-glucose-mannitol [SAGM], 500 mL, Bactivam LQT 7290LX; Lille, France) or by Baxter (4-OptiPure RC CPD plus SAGM quadruple blood collection system, CRGR8448b; Round Lake, IL). Collected whole blood units (480 mL target volume) were rapidly cooled on 1,4-butanediol plates (CompoCool, Fresenius-Kabi, Bothell, WA) and held overnight at room temperature. On the next day, each unit was centrifuged (3496 \times g, accumulated centrifugal force of 7.83 \times 10^5 \times g \text{ min}) and transferred to a semiautomated extracting device (Compomat G4, Fresenius-Kabi) to separate the three layers: PLT-poor plasma, BC, and RBC. The PLT-poor plasma and the RBCs were simultaneously expressed into the satellite bags attached to the top and the bottom of the collection container, respectively. For production of PLTs from BC, the Compomat G4 was set to achieve a BC volume of 50 ± 5 mL with a hematocrit (Hct) of 50 percent. The BC components were left to rest for a minimum of 2 hours at 18 to 24°C. Four ABO-matched BC components, a plasma unit from one of the four donors and a PLT storage set were sterile docked together in a series ("train\) fashion) with the plasma unit at the top and the PLT storage bag at the bottom. Irrespective of the source of the collection set, all PLT concentrates were prepared using the same PLT concentrate pooling-and-storage bag system (OptiPure PL2410, Baxter). The height of the train was adjusted to permit the BC bags to hang vertically. The welds between the BC units were opened, the contents were drained into the bottom bag, and then clamps were placed between each bag. The weld between the plasma and the first BC bag was then opened and half of the plasma was released into the first BC bag with the clamp between it and the second bag closed. Gentle massaging was applied to provide adequate mixing, and the procedure was repeated with each subsequent bag. Gradually all attached bags were rinsed to remove residual cells by passing plasma through them. All the material accumulated in the bottom bag, resulting in a pooled BC component.

The pooled BC components were centrifuged using a soft spin (1258 \times g for 5 min) to separate the PRP from the residual cells. For production of PLTs from BC, the Compomat G4 was set to achieve a BC volume of 50 ± 5 mL with a Hct of 50 percent. The semiautomated extractor expressed the PRP from the pooled BC through a leukoreduction filter and into the PLT storage bag. The BC-PCs as a final product were weighed, labeled, and stored on a PLT agitator at 20 to 24°C.

A total of 24 units (12 for each brand of collection set) were analyzed for PLT quality variables on the day of PLT production (Day 1) and on Day 6 of storage. Conventional quality assurance tests included pH, PLT count, product volume, residual WBC count, and sterility. Additional testing that assesses the metabolic condition and activation status of the PLTs was performed to ensure a better understanding of the product quality; tests included metabolic variables (glucose, lactate, pO2, pCO2), morphology score, PLT activation marker CD62, HSR, and extent of shape change (ESC) in response to adenosine 5′-diphosphate (ADP).

Sampling for testing

Sterility was monitored using a bacterial detection system (BacT/ALERT 3D system, bioMérieux, Durham, NC). On Day 1, the sample pouch was used as a source of PLTs, and for Day 6, a sample was obtained from the PLT storage bag using a sampling site coupler with an 18-gauge needle. By use of sterile technique, each bag was sampled to determine bacterial contamination (8 mL into an aerobic BacT/ALERT bottle), the laboratory test panel (10-mL conical tube), blood gas (hermetically sealed 1-mL syringe), and cell count (3 mL into K3 ethylenediaminetetraacetate tube, Vacutainer, Becton-Dickinson, Franklin Lakes NJ). For
residual WBC enumeration, samples from each bag were fixed with WBC stabilizing solution (Pallfix, Pall Biomedical, Port Washington, NY) before further analysis.

**PLT counts**

PLT count and mean PLT volume were obtained using a cell hematology analyzer (Advia 120, Siemens, Tarrytown, NY). Count and volume were calculated by using the weight of the unit minus the tare weight of the bag and multiplying the result by 1.03 (specific gravity of the PLT concentrate). These values in turn were used to calculate the PLT yield or the total PLT count in the unit.

**Sterility**

Within 1 hour of sampling, the inoculated BacT/ALERT culture bottle was inserted into the BacT/ALERT for sterility testing over a 6-day storage period. In addition, tubing containing PLT concentrate at expiry was sent for sterility testing to NucroTechnics (Scarborough, Ontario, Canada), an outside contractor providing sterility testing services to Canadian Blood Services.

**Residual WBC counts**

Of pooled BC-PCs produced for validation, 450 mL of PLTs was fixed with Pallfix, and residual WBCs were enumerated using a WBC counting kit (LeukoSure, Beckman Coulter, Fullerton, CA). Briefly, 100 µL of fixed sample was treated with lysing solution to eliminate RBCs; residual WBCs were stained with propidium iodide, a fluorescent intercalating stain. After 15 minutes’ incubation in the dark, 100 µL of reference beads of known concentration was added to the mixture. Samples were analyzed by flow cytometry (Epics-XL-MCL, Beckman Coulter). The absolute residual WBC count was calculated according to the manufacturer’s instructions.

**PLT metabolic measurements**

Using aseptic technique, a 1-mL sample was drawn from the sample pouch on Day 1 or through a sampling site coupler from the PLT bag on Day 6. The syringe was sealed and analyzed within 30 minutes of sampling using a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Orangeburg, NY), which reports measurements of pH, pO2, pCO2, glucose, and lactate. For glucose and lactate results exceeding the normal range a 1:2 dilution of the sample was made with phosphate-buffered saline (PBS), and the test was repeated. Tests were performed in duplicate.

**PLT activation**

The expression of PLT surface P-selectin (CD62), a degranulation marker, and the pan-PLT marker CD42a (glycoprotein Ib/IX) were detected by flow cytometry as previously described. Antibodies used for this study included monoclonal fluorescein isothiocyanate–labeled antibody CD42, phycoerythrin-labeled CD62, and appropriate isotype controls (Immunotech, Marseille, France). Flow cytometric analysis was performed using a flow cytometer (Epics XL-MCL, Beckman Coulter). The PLT population was defined based on forward-scatter and side-scatter characteristics. Events with higher fluorescence than the preset 2 percent negative control were considered to be positive for CD62.

**Morphology score**

The morphology of BC-PCs was assessed by modified Kunicki morphology scoring. One-hundred microliters of PLT concentrate was treated with an equal volume of 4 percent paraformaldehyde. Samples were stored at room temperature and analyzed within 1 week by light microscopy with a 1000× oil immersion lens, phase contrast 3 (Nikon, Mississauga, Ontario, Canada). One-hundred PLTs in total were counted and categorized by shape as discoid, spiny sphere, or balloons. The morphology score was calculated by multiplying the number of discoid PLTs by 4, spiny spheres by 2, and balloons by 1 and then adding them together.

**ESC and HSR**

ESC and HSR assays were used to assess PLT quality during storage and reflect the PLT responsiveness to activation with agonist (ADP) or stress (hypotonic shock). These measures were made by light scattering assessment using an aggregometer (SPA 2000, Chronolog Corp., Havertown, PA) according to Holme and coworkers. Both assays were performed at 37°C. PLT concentration was adjusted to $300 \times 10^9$ per L using N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid)–buffered autologous PLT-poor plasma obtained by centrifugation of 5 mL of PLT concentrate at $2060 \times g$ for 20 minutes. The pH value of the plasma was adjusted to 7.0 to 7.4. The ESC was calculated by the instrument by integrating the optical density reading from PLT-poor plasma, PRP with no ADP and PRP after addition of ADP (20 µmol/L final concentration). For HSR, the PLT response to PBS (dilution response) and water (PLT swelling) was recorded and measured as a recovery at 1 and at 4 minutes. The normal HSR is reported to be between 50 and 90 percent.

**Standard QC measurement**

The routine QC in Canadian centers includes monthly measurement of pH, PLT volume and count, sterility, and residual WBC count. This QC was performed using a standard pH meter (Omega pHH-1000, Omega Engineering,
Stamford, CT), the Advia 120 for PLT count, BacT/ALERT 3D for sterility testing, and residual WBC by flow cytometry as described above. We collected QC data for PRP-PCs produced by the first two production sites that converted to BC production for the 12 months before conversion. These PRP units were prepared using a RC-PL system (Pall Biomedical). After implementation, the routine QC for BC production was collected after implementation for a period of 26 months in the first site and 5 months in the second site.

**Statistical analysis**

All quantitative results are expressed as mean and standard deviation (SD). The statistical calculations were performed using computer software (GraphPad InStat, GraphPad Software, Inc., San Diego, CA). The differences between Day 1 and Day 6 of PLTs produced for validation and between PRP and BC-PCs was established by t test. p Values of less than 0.05 were considered to be significant.

**RESULTS**

The variables of BC-PCs produced during process validation are shown in Table 1 and summarize the PLT condition on the day of production (Day 1) and on Day 6 of storage. All PLT products were manufactured using a single type of PLT storage container. On Day 1, irrespective of the manufacturer of the primary blood collection set, all tested units met the standards of the Council of Europe’s Guide to the Preparation, Use and Quality Assurance of Blood Components as well as the manufacturers’ specifications of PLT count per unit (≥1.5 × 10¹¹ and ≤4.5 × 10¹¹ PLTs/unit in all units). Thus, for purposes of analysis, the data sets from the two different primary collection set manufacturers were pooled. Over the storage period, the absolute PLT concentration in BC-PCs remained the same but the total PLT count decreased by approximately 10 percent as a result of the samples drawn for analysis on Day 1.

No bacterial growth was detected by any testing method in any of the PLT concentrates manufactured from BCs. In North America, the QC criterion for residual WBC content following leukoreduction is set at fewer than 5 × 10⁶ WBCs per unit. Adequate leukoreduction was achieved (Table 1) in all units with a mean residual WBC count of 0.5 × 10⁵ ± 1.7 × 10⁵ WBCs per unit. pH was well maintained during storage with no decrease of pH observed. In fact, pH continued to rise slightly over time. As expected, glucose was depleted over the storage period with a concomitant production of lactate (Table 2).

Consistent with the development of the PLT storage lesion, the PLT morphology score on Day 6 of storage was significantly lower than on Day 1 (Table 2) and negatively correlated (r² = -0.62) with PLT activation marker CD62.

<table>
<thead>
<tr>
<th>Test (n = 24)</th>
<th>Day 1</th>
<th>Day 6</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT count (x10⁹/L)</td>
<td>870.88 ± 113.41 (636.00-1120.00)</td>
<td>816.83 ± 99.05 (589.00-1023.00)</td>
<td>0.085</td>
</tr>
<tr>
<td>PLT yield (x10⁹/unit)</td>
<td>309.3 ± 36.1 (240.6-381.6)</td>
<td>264.7 ± 27.6 (207.3-313.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>267.40 ± 100.06 (94.17-381.07)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>pH</td>
<td>7.05 ± 0.02</td>
<td>7.28 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual WBCs Cells/mL</td>
<td>0.15 ± 0.46 (0.00-2.30)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Residual WBCs x10⁵/unit</td>
<td>0.5 ± 1.7 (0.00-8.66)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Data are reported as mean ± SD (range).
† Difference between Day 1 and Day 6 determined by t test.
NA = not applicable.

<table>
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<th>Test (n = 24)</th>
<th>Day 1</th>
<th>Day 6</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology score</td>
<td>302.7 ± 15.3 (267.50-331.50)</td>
<td>234.6 ± 9.1 (220.00-255.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%CD62 expression</td>
<td>21.21 ± 5.45 (12.10-33.30)</td>
<td>31.57 ± 9.44 (15.54-47.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESC</td>
<td>27.00 ± 5.30 (19.40-41.00)</td>
<td>27.21 ± 3.74 (20.54-47.20)</td>
<td>0.636</td>
</tr>
<tr>
<td>HSR</td>
<td>61.58 ± 8.58 (50.30-77.65)</td>
<td>66.12 ± 9.67 (53.70-93.40)</td>
<td>0.092</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>8.07 ± 0.79 (6.45-9.20)</td>
<td>13.36 ± 1.24 (10.60-15.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>17.03 ± 0.86 (15.85-19.40)</td>
<td>14.52 ± 0.96 (12.95-16.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>88.19 ± 12.58</td>
<td>100.52 ± 10.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>71.69 ± 4.48</td>
<td>27.19 ± 2.54</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Data are reported as mean ± SD.
† Difference between Day 1 and Day 6 determined by t test.
expression. There was no significant change of PLT responsiveness to stimulation with ADP (ESC) or to hypotonic shock (HSR).

To assess the characteristics of the two PLT components in a more robust setting than is achieved in a controlled validation exercise, we compared the QC results from the first two Canadian Blood Services production sites to implement BC as part of routine PLT component production to their results for PRP-PCs immediately before BC implementation. The QC testing results from these two manufacturing facilities are shown in Table 3. Because the same standard operating procedures are used in both locations, data were pooled across the two facilities. In comparison to PRP-PCs, concentrates produced by the BC method generally had a higher pH value on Day 6. Owing to the differences of the production method, the PLT concentration in BC products was somewhat more dilute than in PRP-PCs. In other aspects, the QC variables were not different between the two methods.

**DISCUSSION**

This study reports the early experience during conversion to PLT production by the BC method at Canadian Blood Services. Consistent with findings in the literature, the pH of the BC-produced PLTs was higher than that in PRP-PCs. It was reported previously by Murphy that BC-PCs store better due to reduced glycolysis, increased oxidative metabolism, and better maintenance of bicarbonate levels and, therefore, pH. During validation, we performed additional testing that is conventionally believed to reflect PLT quality and its change during storage. These tests, including expression of the PLT activation marker CD62, ESC, and morphology scoring have been reported to correlate with PLT survival after transfusion. The change in CD62 expression between Day 1 and Day 6 reflects ongoing PLT activation in the storage container. Although the increase over the storage period was significant for BC-PCs, it was far less than increases by outdate reported in the literature for PRP-PCs (approx. 30% for BC-PCs in this study compared to 40%-70% in the literature for PRP-PCs). Similarly, although the morphology score decreased somewhat over the storage period, it remained well above 200, suggesting that most of the PLTs retained a discoid shape throughout the storage period. Interestingly, neither the HSR nor the ESC showed sensitivity to time in storage. In the literature, the response of this assay to the effect of storage is variable, which may reflect inconsistency among the protocols used by different laboratories.

There is considerable interest in the comparison between BC-PCs and PRP-produced PLTs. The full conversion of two Canadian Blood Services centers to BC manufacture allowed the comparison of PRP-PC QC results for 12 months before implementation to the QC data for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PRP-PCs</th>
<th>BC-PCs</th>
<th>p Value (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>&gt;6.2</td>
<td>6.6-7.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>NA</td>
<td>10% of labeled volume</td>
<td>0.0001</td>
</tr>
<tr>
<td>PLT count (x10^9/L)</td>
<td>401.0 (329.9-1300.0) (n = 843)</td>
<td>882.2 (543-1100) (n = 349)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PLT yield (x10^9/unit)</td>
<td>&gt;240 x 10^9 PLTs/unit in 75% of units tested</td>
<td>&gt;75% of units tested</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual WBCs (x10^5/unit)</td>
<td>&lt;8.3</td>
<td>&lt;50 x 10^5/10^9 PLTs/unit in 75% of units tested</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**TABLE 3. Comparison of QC variables for PLTs produced by PRP and BC methods**

*CSA = Canadian Standards Association; NA = not applicable.*
BC-PCs after implementation. These comparisons were made using the standard QC measures of the blood center rather than the more sophisticated tests performed during validation. Leukoreduction was more effective in the BC-PC products. Although the overall mean WBC count in BC-PCs on a “per-unit” basis was the same as in the PRP-PCs (Table 3), the typical transfusion dose of 5 PRP-PC units means a greater number of WBCs would be transfused to the recipient. Of note, the Pall leukoreduction filter in the RC-PL system is designed to meet the North American standard of fewer than $8.3 \times 10^6$ per unit (<5 × 10⁶ per transfusion dose) whereas the Baxter filter is designed to meet the European standard of fewer than 1 × 10⁶ per unit.

Compared to PRP-PCs, the BC method of PLT production offers significant advantages to the hospital because it is a prestorage pooled product that can be bacterially tested by the manufacturer. For the manufacturer, it is a cost-effective method, in part owing to the increased recovery of plasma and in part to a 24-hour production window, which allows all manufacturing to be done on the day after collection. In addition, the use of semiautomated production equipment allows better process control and more efficient use of staff. Although there are relatively few in vivo data to support the suggestion that the BC-PC is a superior product to the PRP-PC, the data reported here are consistent with a number of in vitro studies in the literature, suggesting that this type of PLT product is superior.⁴,⁵,⁶ On balance, with the aforementioned benefits to both the patients and the manufacturers of whole blood–derived PLTs, this is a method that has proven its value in non–North American jurisdictions and has now become the production method of choice in Canada.

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