The use of new generation small-volume blood collection tubes for complete blood count

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Abstract

Objectives: Newborns and especially patients with malignancy develop frequently iatrogenic anemia based on phlebotomy. The use of blood collection tubes with small-volume may reduce the need for blood transfusion and the associated risks due to frequent phlebotomy. Therefore, we evaluated the reliability and accuracy of the complete blood count (CBC) using new generation small-volume blood collection tubes (SV-BCT) instead of large-volume blood collection tubes (LV-BCT).

Methods: Venous blood samples were taken from 40 adult in-patients and collected to SV-BCT/LV-BCT pairs of three different brand (Microtainer®MAP-0.5 ml/Vacutainer®-2.0 ml; Becton, Dickinson and Company, USA) ([Microvette®-0.5 ml/ Monovette®-2.6 ml; Sarstedt Ag and Co. KG, Germany]) ([MiniCollect®Complete-0.5 ml/Vacuette®-2.0 ml; Greiner Bio-One GmbH, Austria]). All tubes contained K2EDTA except Microvette®. Sixteen parameters of CBC were analyzed using a DxH 800 (Beckman Coulter Inc., USA). CBC results in tube pairs were compared in terms of statistical and clinical (bias%) significance.

Results: There were statistically significant differences between the results of SV-BCT and LV-BCT pairs of the same brands for some parameters. However, bias% between tubes for 16 parameters was within the desirable limits, the differences were not clinically significant.

Conclusion: Health personnel and phlebotomists can safely prefer SV-BCT which is a new generation and technically useful for CBC, especially in patients requiring frequent phlebotomy. Thus, the volume of blood sampling may be reduced to prevent iatrogenic blood loss.

Keywords: Blood collection tube, complete blood count, iatrogenic anemia, phlebotomy

years [3, 4]. In addition, it is shown that costs, length of stay, and need for transfusion are significantly decreased by reducing the number of ordered unnecessary laboratory tests [5].

Pediatric small-volume blood collection tubes (SV-BCT) were produced in the late 1970s due to their advantages in patients who had difficulty in collecting blood samples and required frequent blood sampling. Nevertheless, pediatric blood sampling tubes were not suitable for directly use in auto analyzers. There were some obstacles to the widespread use of SV-BCT. SV-BCT had disadvantages such as their labeling difficulties, and manual loading and running to the devices. In addition, it could rise injury risk, loss of time, and over workload. The innovations such as manufacturing SV-BCT or micro-collection tubes, we believe those disadvantages no longer exist.

Collecting of blood samples
Venous blood samples were collected in three different brand tubes (six tubes overall) both SV-BCT and LV-BCT tubes within 5 consecutive days, and all samples were simultaneously analyzed on the collection day.

Materials and Methods
Participants
A total of 40 adult inpatients at Internal Medicine Clinics were randomly selected in this study. As possible, especially in patients with different chronic diseases (diabetes mellitus, hyperlipidemia, anemia, blood cancer, thyroid, liver disorders etc.) were included in the study to obtain data covering the clinical decision limits or analytical range for each test simultaneously. This analytical comparison study conducted under the Helsinki Declaration [12] and with the approval of the local ethics committee. Informed consent obtained from the patients.

Blood collection tubes
SV-BCT and LV-BCT of the same brand were compared. Becton, Dickinson, and Company (BD) and Greiner Bio-One GmbH (GBO) tube pairs contained K2EDTA, while Sarstedt AG and Co tube pairs have K3EDTA. All tubes have been validated by the manufacturers. It was considered that the tube pairs have the same EDTA content and that would not create an obstacle for peer-to-peer comparison. All tubes are as follows;
   a. Vacutainer® (Reference number: 367842, 2 ml, 13×75 mm) and Microtainer®MAP (Reference number: 363706, 0.5 mL, 13×75 mm); (Becton, Dickinson and Company (BD), NJ, USA).
   b. Vacuette® (Item No.: 454047, 2 ml, 13×75 mm) and MiniCollect®Complete (Item No.: 450547, 0.25–0.5 ml, 13×75 mm); (GBO GmbH, Kremsmünster, Austria).
   c. 5-Monovette® (Order Number: 04.1901.100, 2.6 ml, 13×65 mm) and Microvette® (Order number: 20.1341.100, 0.5 mL, 10.8×47.6 mm); (SARSTEDT AG & Co. KG, Nümbrecht, Germany). External tube adapter (13×75 mm) used for Microvette® tubes.

CBC parameters and its analysis
White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), platelet (PLT), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), MCH concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), neutrophil (NE), lymphocyte (LYM), monocyte (MO), eosinophil (EO), basophil (BA), and plateletcrit (Pct) were analyzed by an automated hematology analyzer using impedance and multi-angle laser scatter method (DxH 800, Beckman Coulter Inc., USA). The commercial internal and external quality control materials were run to ensure the reliability of test results throughout the study.

All blood samples were run on the same analyzer. To simultaneously analyze CBC within six different blood collection tubes, they were automatically loaded using a rack system to an automated blood counter device. Blood samples were run in a random order in duplicate.

Statistical analysis
Statistical analysis was performed using MedCalc statistical program (MedCalc Software, Belgium). The duplicate results were averaged. The mean values between the tube pairs were statistically and clinically compared. The normal distribution of data was analyzed using Shapiro–Wilk test.
The results were represented as mean ± standard deviation for normally distributed data or median (minimum-maximum) for non-normal distributed data. *: Statistically significant difference between tubes was considered as p<0.05. P value of the correlation coefficient for all parameters between paired tube was r=0.800, p<0.001.

Table 1. Comparison results of BD brand tubes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vacutainer*</th>
<th>Microtainer* MAP</th>
<th>r</th>
<th>bias%</th>
<th>biasd%</th>
<th>p</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10^9/L)</td>
<td>7.7 (3.3–19.7)</td>
<td>7.7 (3.3–19.7)</td>
<td>0.998</td>
<td>-0.41</td>
<td>4.59</td>
<td>0.210</td>
<td>0.992–1.008</td>
<td>-0.075–0.056</td>
</tr>
<tr>
<td>RBC (×10^12/L)</td>
<td>3.95±0.87</td>
<td>3.97±0.88</td>
<td>0.998</td>
<td>0.52</td>
<td>1.56</td>
<td>0.005*</td>
<td>0.987–1.021</td>
<td>-0.058–0.081</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>106 (70–148)</td>
<td>106 (71–150)</td>
<td>0.997</td>
<td>0.46</td>
<td>1.61</td>
<td>0.005*</td>
<td>1.000–1.037</td>
<td>-0.309–0.005</td>
</tr>
<tr>
<td>PLT (×10^9/L)</td>
<td>106 (75–735.5)</td>
<td>211 (71–738.5)</td>
<td>0.997</td>
<td>-1.34</td>
<td>2.55</td>
<td>0.003*</td>
<td>0.970–1.015</td>
<td>-7.251–4.989</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>32.7±6.5</td>
<td>32.9±6.5</td>
<td>0.998</td>
<td>0.46</td>
<td>1.34</td>
<td>0.045*</td>
<td>0.978–1.014</td>
<td>-0.212–0.016</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>584±9.3</td>
<td>839±9.4</td>
<td>0.999</td>
<td>-0.24</td>
<td>1.01</td>
<td>0.018*</td>
<td>0.995–1.031</td>
<td>-2.688–0.258</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.3±3.9</td>
<td>28.2±4.0</td>
<td>0.998</td>
<td>-0.04</td>
<td>0.75</td>
<td>0.006*</td>
<td>0.987–1.029</td>
<td>-0.062–0.267</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>330±14</td>
<td>336±15</td>
<td>0.967</td>
<td>-0.14</td>
<td>0.47</td>
<td>0.429</td>
<td>0.957–1.150</td>
<td>-0.085–1.149</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.1 (12–37.6)</td>
<td>15.0 (12.2–37.5)</td>
<td>0.992</td>
<td>0.01</td>
<td>0.69</td>
<td>0.915</td>
<td>0.981–1.004</td>
<td>-0.057–0.331</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>9±1</td>
<td>8±1</td>
<td>0.990</td>
<td>0.16</td>
<td>1.54</td>
<td>0.660</td>
<td>0.951–1.031</td>
<td>-0.027–0.430</td>
</tr>
<tr>
<td>NE (×10^9/L)</td>
<td>4.9 (1.3–18)</td>
<td>4.9 (1.3–18)</td>
<td>0.998</td>
<td>-0.21</td>
<td>5.08</td>
<td>0.519</td>
<td>0.986–1.015</td>
<td>-0.083–0.057</td>
</tr>
<tr>
<td>LYM (×10^9/L)</td>
<td>1.6±0.6</td>
<td>1.6±0.6</td>
<td>0.996</td>
<td>-0.75</td>
<td>5.65</td>
<td>0.343</td>
<td>1.000–1.000</td>
<td>0.000–0.000</td>
</tr>
<tr>
<td>MO (×10^9/L)</td>
<td>0.6 (0.1–1.6)</td>
<td>0.6 (0.15–1.6)</td>
<td>0.962</td>
<td>-1.01</td>
<td>5.07</td>
<td>0.083</td>
<td>1.000–1.000</td>
<td>0.000–0.000</td>
</tr>
<tr>
<td>EO (×10^9/L)</td>
<td>0.1 (0.0–0.6)</td>
<td>0.1 (0.0–0.6)</td>
<td>0.978</td>
<td>-4.42</td>
<td>17.8</td>
<td>0.564</td>
<td>1.000–1.000</td>
<td>0.000–0.000</td>
</tr>
<tr>
<td>BA (×10^9/L)</td>
<td>0.0 (0.0–0.1)</td>
<td>0.0 (0.0–0.1)</td>
<td>0.573</td>
<td>-1.25</td>
<td>6.21</td>
<td>0.531</td>
<td>0.500–2.000</td>
<td>0.000–0.000</td>
</tr>
<tr>
<td>Pct (%)</td>
<td>0.02 (0.07–0.57)</td>
<td>0.21 (0.07–0.55)</td>
<td>0.988</td>
<td>-1.07</td>
<td>2.97</td>
<td>0.004*</td>
<td>0.954–1.000</td>
<td>-0.005–0.007</td>
</tr>
</tbody>
</table>

The results were generated to ascertain areas of bias between paired tubes. The mean difference was also assessed with Bland-Altman plots and then compared with the bias.

Results

When we compared SV-BCT and LV-BCT of each brand; RBC, Hb, PLT, Hct, MCV, MCH, and Pct levels in BD (Table 1); MCV and MCH levels in GBO (Table 2); WBC, RBC, PLT, MCV, NE, and Pct levels in Sarstedt (Table 3) were found statistically different. Correlation coefficients between all paired tubes were greater than 0.900 for all parameters, except BA. For BA, while the relationship between paired tubes of BD was found moderate (r=0.573, p<0.01), GBO and Sarstedt paired tubes had high relationships (r=0.683, p<0.001 and r=0.800, p<0.001).

According to the regression analysis, systematic, and proportional errors were detected for PLT in GBO tubes. PLT in Vacuette® were statistically higher than in MiniCollect® Complete, but the difference was not clinically significant (Table 2). In Sarstedt tubes, there was systematic error for RBC, Hb, and Hct; both systematic and proportional errors were detected for MCHC parameters (Table 3). Only Hb and PLT in S-Monovette® were statistically less than in Mircrovette®, but not clinically significant. However, bias% values between SV-BCT and LV-BCT of three different brands for all parameters were within desirable limits (Tables 1-3), and the clinical difference (based on the EuBiVAS) was not found [15].

Bland-Altman plots comparing the difference between the results of paired tubes were shown in Figures 1-3, respectively, except for EO and BA parameters. Since the mean of EO and BA were zero, the difference as % of the mean could not be expressed in the Bland-Altman charts. In general, bias% between paired tubes was decreased by the increasing values of all parameters. It was seen bias% values up to 20% in NE, MO, LYM, PI, and Pct values, up to 8% in RBC, Hb, Hct, and WBC values, and up to 3% in MCV, MCH, and MCHC values. However, the mean bias% values were not exceeded 2%.
Discussion

Although there are some studies where different SV-BCT were compared or validated [16–20], our study is the first to compare SV-BCT and LV-BCT of three different brands in automated blood counter analyzers under the same conditions. The crucial result of this comparison is that CBC results in SV-BCT on automated blood-counting devices may be clinically reliable. In our study, the new generation SV-BCT is automatically processed, centrifuged, and labeled like a standard large-
volume tube. It fits in instrument racks, thus, transferring to a secondary tube or manual pipetting is eliminated.

When the pairs of SV-BCT and LV-BCT were compared, the adaptation of the existing SV-BCT to the automated systems would be advantageous for both the patient and laboratorian. By reducing blood loss due to phlebotomy, the risk of iatrogenic anemia, the need for transfusion, and the resulting complications and cost burden may be reduced [21]. It has also been shown that reducing the amount of blood taken from patients shortens the length of hospital stay [22–24]. Thus, instead of dealing with side problems that may arise from phlebotomy, clinicians will be able to deal more with the patient and other issues related to the disease during hospitalization. As the blood volume taken from the patients will decrease by approximately 75% (0.5 mL/2.0 mL), it can be estimated that the rate and cost of medical waste will decrease. In a recent study, a cost analysis was performed over an infant who was hospitalized for 40 days. During the period, 2 mL of blood was taken from the baby for each CBC and a total of 4 units of erythrocyte suspension were given for a total of 26 mL of blood loss. When the costs of the erythrocyte suspension and blood collection tubes were calculated together and the reduction in expenses was tried to be estimated according to the estimated erythrocyte suspension need in case of using a pediatric tube. Accordingly, it has been calculated that using SV-BCT instead of LV-BCT can cost almost 74% reduced cost in total [4].

The new generation SV-BCT for the automated process is the one-piece microtube to offer compatibility with most automated blood counter devices. These SV-BCT offer the extra advantage of having the carrier tube permanently attached, fur-
ther reducing the need for separate accessories. They can be automatically handled, processed, centrifuged, and labelled as a primary tube as a standard LV-BCT. They fit in instrument racks and eliminate the need for manual processing. Thus, extra procedures such as transfer to a secondary tube or manual pipetting will be eliminated. This is an opportunity and convenience for a large-scale clinical laboratory. One can speculate that employee safety can increase by automating laboratory tests compared to manual work on analyzers. In addition to these speculations, reduced medical waste or decreased cost would be among the possible positive outcomes of the study.

In addition to the advantages of SV-BCT, it few disadvantages, such as being vacuum-free, and not being able to run a test a 3rd time. However, running a CBC a 3rd time is a rare situation according to our clinical experiences. We can strongly recommend the use of these tubes, in patients (especially in newborns, pediatric oncology and hematology, and other pediatric units) collecting blood with the needle tip or syringe. SV-BCT will provide benefit when the use of tubes with vacuum is limited, especially due to structural vascular disorders, etc.

**Limitations**

In our study, patients from the Internal Medicine service were included in the study so that the outcome range of the parameters could be particularly wide. However, due to ethical concerns, blood was not drawn from patients with extreme CBC values. In addition, the advantages of SV-BCT in terms of patients, employees, costs, and medical waste could not be determined and remained speculative. A long-term study with more detailed plan can address these limitations.
Conclusion

New generation SV-BCT can be automatically processed, centrifuged, and labeled like a standard large-volume tube. It fits in instrument racks, thus, transferring to a secondary tube or manual pipetting will be eliminated.

However, clinical validation is required to prove that SV-BCT does not compromises on quality. This clinical validation study can contribute to advancing knowledge about SV-BCT, so to widely use of these.

Iatrogenic anemia and its risks may be prevented by reducing the blood volume collected for CBC. These tubes may provide convenience for phlebotomists who use a needle or syringe. The safety of laboratory staff may be increased by removing the manual process, such as transferring the sample to another tube.

We showed that the SV-BCT can be safely used instead of the LV-BCT of the same brand. Today, in an era when the test systems can be worked with a drop of blood samples and the test adapters developed for mobile phones are discussed, it is not considered sustainable for patients and employees to perform blood testing with such high sample volumes. We also recommend developing new blood tubes suitable for automated systems and demonstrating their clinical relevance to encourage the use of SV-BCT for other laboratory tests.

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Conflict of Interest: The authors declare that there is no conflict of interest.
Ethics Committee Approval: The study was approved by The Dokuz Eylül University Non-interventional Research Ethics Committee (No: 2016/26-32, Date: 06/10/2016).

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