Comparison of Low Volume and Conventional Sodium Citrate Tubes for Routine Coagulation Testing

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SUMMARY

Background: Low-volume sample tubes reduce unnecessary blood loss due to repeated blood collection and are more convenient to collect blood from patients with difficult veins. However, different sample tubes may be sources of preanalytical bias, and the corresponding test results may reflect clinically important differences. We compared the new low-volume sodium citrate tube to the conventional sodium citrate tube to determine any significant differences between the two types of tubes for routine coagulation testing.

Methods: We collected blood samples from 48 random patients, who were referred for routine coagulation testing, into low-volume (1 mL) and conventional (2.7 mL) sodium citrate tubes. We assayed the samples for prothrombin time (PT), activated partial thromboplastin time (aPTT), and D-dimer using the automated coagulation analyzer.

Results: There was excellent correlation (r > 0.97) between the results of the two tubes for PT, aPTT, and D-dimer. The PT and aPTT for the low-volume sodium citrate tube were significantly shorter than those of the conventional sodium citrate tube. There was no statistically significant difference in the results for D-dimer. The percent biases calculated with Bland-Altman analysis were 0.8% for PT and 2.0% for aPTT. Both of them were within the desirable specifications for bias with 2.0% for PT and 2.3% for aPTT.

Conclusions: It is imperative to perform local validation before introducing new sodium citrate tubes into the routine blood collection practice. Every laboratory needs to standardize the procedures for evaluation of these tubes.

KEY WORDS

coagulation testing, low-volume sodium citrate tube, validation

INTRODUCTION

The development and successful implementation of high-quality analytical standards have diminished the influence of analytical errors on the reliability of laboratory diagnostics [1]. Preanalytical activities, especially blood sample collection, are the most vulnerable steps during the laboratory testing process [2]. Despite growing evidence of preanalytical bias, blood collection devices in healthcare facilities are often overlooked; hence, tenders are frequently determined by cost rather than the quality of devices [3]. This may be due to the lack of awareness about the importance of preanalytical quality management and blood collection process as
MATERIALS AND METHODS

We obtained blood samples from 48 random patients referred for routine coagulation testing to the department of Laboratory Medicine, Kyungpook National University Hospital. An expert phlebotomist collected a blood sample from each patient and transferred it into two different sodium citrate tubes: conventional Vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA) and low-volume MiniCollect (Greiner Bio-One GmbH, Kremsmünster, Austria). Table 1 displays the characteristics of the blood collection tubes. All collected tubes were centrifuged at 1,500 x g for 15 minutes at room temperature. The overall time between sample collection and testing was less than 1 hour.

We assayed PT and aPTT using the Thromborel S and Actin FSL reagents (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), respectively, and the fully automated CS-5100 coagulation analyzer (Sysmex Corporation, Kobe, Japan). D-dimer was measured using the STA-Liatest D-Di assay kit (Diagnostica Stago, Asnières sur Seine, France) and the STA Compact Max3 analyzer (Diagnostica Stago, Asnières sur Seine, France). The manufacturers’ instructions were followed for all the assays.

Statistical analysis
Test results were expressed as mean values with standard deviation. Pearson’s correlation coefficient was calculated to correlate the results of the two types of collection tubes. A paired t-test was performed to compare the results, and a p-value below 0.05 was considered statistically significant. For the results with statistically significant differences, a Bland-Altman plot was generated to evaluate the clinical agreement between the test results [10]. The percent bias was calculated with Bland-Altman analysis to compare with the desirable specifications for a bias of 2.0% for PT and 2.3% for aPTT, as described by Ricós et al. [11,12]. All statistical analyses were performed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA).

RESULTS

Table 2 displays the results of PT, aPTT, and D-dimer conducted in two types of sodium citrate tubes. The Pearson correlation coefficients for both tubes were excellent (r > 0.97) for PT, aPTT, and D-dimer assay results. Figure 1 represents the linear correlation analyses. The PT and aPTT of samples from the low-volume MiniCollect tube were significantly shorter than those from the conventional Vacutainer tube. The D-dimer levels of samples from the low-volume MiniCollect tube were higher than those from the conventional Vacutainer tube; however, this difference was not statistically significant. The percent biases calculated with Bland-Altman analysis were 0.8% and 2.0% for PT and aPTT, respectively, with both within the desirable specifications for bias. Figure 2 displays the Bland-Altman plots indicating good agreement between the PT and aPTT results from the two different sodium citrate tubes.

DISCUSSION

Our study concluded that there was an excellent correlation and agreement between the results from the low-volume and conventional sodium citrate tubes for routine coagulation tests, including PT, aPTT, and D-dimer. Additionally, our results revealed that the low-volume sodium citrate tubes might influence certain routine coagulation tests, such as PT and aPTT. Adam et al. compared a small-volume (1.8 mL) sodium citrate tube with a conventional (3 mL) sodium citrate tube for coagulation parameters, including PT, international normalized ratio (INR), aPTT, thrombin clotting time, fibrinogen, antithrombin, and D-dimer, in critically ill patients [13]. The results showed that both tubes were interchangeable with good correlation (r > 0.96) and agreement for all the tests. Önlöv et al. reported that there was no difference in the results of PT/INR for venous samples, collected using a 1-mL sodium citrate tube in comparison with the conventional 4.5-mL sodium citrate tube [14]. On the contrary, studies have reported differences in test results of samples from different sodium citrate
Table 1. Characteristics of blood collection tubes.

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Anticoagulant</th>
<th>Type of tube</th>
<th>Draw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacutainer</td>
<td>Becton Dickinson, Franklin Lakes, NJ, USA</td>
<td>buffered sodium citrate 3.2%</td>
<td>evacuated, 13 x 75 mm</td>
<td>2.7 mL</td>
</tr>
<tr>
<td>MiniCollect</td>
<td>Greiner Bio-One GmbH, Kremsmünster, Austria</td>
<td>buffered sodium citrate 3.2%</td>
<td>nonevacuated, 10 x 43 mm</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

Table 2. Results of PT, aPTT, and D-dimer obtained in different sodium citrate tubes.

<table>
<thead>
<tr>
<th>Test</th>
<th>Vacutainer 2.7 mL</th>
<th>Minicollect 1 mL</th>
<th>r</th>
<th>p-value *</th>
<th>Percent bias (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (s)</td>
<td>23.4 ± 8.8</td>
<td>23.3 ± 8.8</td>
<td>0.999</td>
<td>0.001</td>
<td>0.8% (0.5 to 3.3%)</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>35.7 ± 6.6</td>
<td>35.0 ± 6.9</td>
<td>0.987</td>
<td>&lt; 0.001</td>
<td>2.0% (0.9 to 3.2%)</td>
</tr>
<tr>
<td>D-dimer (mg/L FEU)</td>
<td>0.61 ± 0.78</td>
<td>0.67 ± 1.05</td>
<td>0.976</td>
<td>0.209</td>
<td>NE</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or number.
*p-value < 0.05 was considered statistically significant.
r is the Pearson’s correlation coefficient.
Abbreviations: CI - confidence interval, s - seconds, FEU - fibrinogen equivalent units, NE - not evaluated.

Low Volume Tube for Coagulation Testing

tubes. Salvagno et al. reported that Spearman’s correlation was always satisfactory. However, they frequently observed a statistically significant bias in the PT, aPTT, and fibrinogen among five different sodium citrate tubes, including two conventional evacuated tubes (3 mL and 2 mL), one evacuated low-volume tube (1 mL), and two different nonevacuated microtubes (0.5 mL each) [4]. Lima-Oliveira et al. observed significant differences for the PT and aPTT tests but not for fibrinogen determination in five different sodium citrate tubes, including 1.4-mL, 2.0-mL, 2.7-mL, and two 3.6-mL tubes [5].

Thus, the results varied depending on the researchers and sizes of tubes evaluated. All these results support the need for local (user) validation before introducing new tubes for coagulation testing in the laboratory. The Working group for Preanalytical phase of the European Federation for Clinical Chemistry and Laboratory Medicine suggested that validation of new devices should include statistical analysis of laboratory data obtained with the existing and locally validated blood collection tubes prior to introduction into routine practice [3]. If the analysis results are not acceptable, new tubes should not be used without modified reference ranges. However, there are no precise guidelines on comparing the results and criteria for determining the acceptability of the results.

Desirable quality specifications for imprecision, bias, and total errors derived from biological variation, proposed by Ricós et al. [11], are the most important and useful requirements for quality management in laboratory medicine [12]. In the Bland-Altman plot of our results, one aPTT result was an influential outlier that significantly deviated and affected the statistical analysis for correlation and the p-value (Figure 2). Such a significant difference, even if within the desirable specifications for bias [11,12], makes it possible to verify that the new tube was either used in combination or replaced the existing tube without adjusting the reference values. Low-volume blood collection tubes are increasingly in demand for a number of reasons [4,9]. A previous study reported that using small-volume sodium citrate tubes for routine coagulation analyses reduced the unnecessary diagnostic-related blood loss by about 40% [13]. We considered the low-volume tube as a convenient alternative to conventional tube for collecting blood in difficult circumstances, such as in children. Pediatric evacuated collection tubes (2 mL volume or less) may be more sensitive to variances in the fill-volume than the conventional 5-mL tubes. This could be due to the relatively increased plasma dilution in smaller volume tubes, leading to falsely prolonged PT and INR [15]. Therefore, a validation process may be absolutely essential for low-volume sodium citrate tubes.

In conclusion, our study revealed that the results of routine coagulation tests, especially the PT and aPTT tests, conducted on samples collected in low-volume sodium citrate tubes may differ within the desirable specifications for bias, compared with the conventional sodium citrate tubes. Therefore, it is necessary to perform local validation before introducing new tubes into the routine blood collection practice. Additionally, every laboratory needs to standardize the procedures for evaluation of these collection tubes.
Figure 1. Linear correlation analyses between the results from the conventional Vacutainer and low-volume MiniCollect tubes for PT (A), aPTT (B), and D-dimer (C).
Figure 2. Bland-Altman plots comparing the differences between the results from the conventional Vacutainer and low-volume MiniCollect tubes for PT (A) and aPTT (B). Only the results with significant differences (Table 2) were plotted and presented as percent differences.

Abbreviations: SD - standard deviation.

Declaration of Interest:  
Author has no potential conflicts of interest to declare.

References:


