The order of draw: much ado about nothing?
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SUMMARY
Introduction: The ‘order of draw’ has been advocated since 1982 to reduce the risk of cross-contaminating blood tubes with additives from a previously filled tube.
Methods: We studied 193 patients receiving oral anticoagulation. Multiple tubes were collected in a specific order using the Sarstedt Safety Monovette System. We evaluated the effect of the ‘order of draw’ on the prothrombin time/international normalized ratio (PT/INR) and the activated partial thromboplastin time (APTT) when the citrate tube is drawn as the first tube, second tube or after a heparin, EDTA or serum tube with clot activator.
Results: There was no statistically significant influence on the PT/INR. The same applies for the APTT measured on a citrate tube drawn after a heparin tube. There was a small, but statistically significant bias on the APTT when the citrate tube was drawn as the first tube, after an EDTA tube or after a serum tube with clot activator. We consider this bias (max. 0.2 s) as not clinically significant.
Conclusion: The order of draw has no significant influence on the PT/INR and APTT when measured on a Sarstedt citrate tube filled without the use of a discard tube or after a heparin, EDTA or serum tube with clot activator.

INTRODUCTION
The pre-analytical phase is crucial for good-quality laboratory results. Research has shown that correct blood sample collection and handling is amongst the most vulnerable steps in this precarious phase, with relative error rates going up to 75% and above [1, 2].

To ensure a good-quality pre-analytical phase, guidelines have been set up by a number of organizations such as the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) [3] and the World Health Organization (WHO) [4]. The basic criteria for a correct and safe venepuncture are nearly identical for clinical chemistry and immunochemistry tests as for haemostasis testing. However, some particular aspects are especially important for coagulation testing [5]. This includes the prevention of venous stasis, collection of nonhaemolysed samples, using the correct ‘order of draw’ and the appropriate filling and mixing of the primary collection tubes [6].

In 1982, Calam and Cooper suggested that the ‘order of draw’ of blood into tubes containing additive...
of glass tubes and the development of clot activator and gel separator additives [8]. The current ‘order of draw’ entails the collection of blood cultures first, followed by coagulation tubes (citrate) and then by non-additive, clot activator, sodium heparin, lithium heparin, EDTA, acid citrate dextrose and oxalate/fluoride tubes [3, 4]. Glass nonadditive and plastic serum tubes without a clot activator may be drawn before citrate blood tubes, as no contamination risk exists. Furthermore, if a winged blood collection set is used and the citrate tube is the first to be drawn, a discard tube is to be used to ensure correct filling of the citrate tube.

For a consultation in the outpatient anticoagulation clinic of the Onze-Lieve-Vrouw Hospital (OLVZ) in Aalst, two blood samples are routinely taken. A discard tube is drawn first to ensure correct filling of the following tubes and to prevent any influence from released tissue thromboplastin. Subsequently, a citrate blood tube is drawn for the determination of the prothrombin time (PT) and the international normalized ratio (INR) in the hospital laboratory.

### Table 1. Tube specifications

<table>
<thead>
<tr>
<th>Tube</th>
<th>Length x diameter – volume</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate 3.2%</td>
<td>66 mm x 11 mm – 3.0 mL</td>
<td>05.1165.001</td>
</tr>
<tr>
<td>Lithium heparin gel</td>
<td>90 mm x 13 mm – 4.9 mL</td>
<td>04.1940.001</td>
</tr>
<tr>
<td>Serum gel with clot activator</td>
<td>92 mm x 15 mm – 7.5 mL</td>
<td>01.1602.001</td>
</tr>
<tr>
<td>K&lt;sub&gt;3&lt;/sub&gt;-EDTA</td>
<td>66 mm x 11 mm – 2.7 mL</td>
<td>05.1167.001</td>
</tr>
</tbody>
</table>

EDTA, ethylenediaminetetraacetic acid.

### Table 2. Summary of the results

<table>
<thead>
<tr>
<th>PT (INR)</th>
<th>APTT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>Mean bias (95% CI)</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>2.6 (2.1–3.0)</td>
</tr>
<tr>
<td>(n = 95)</td>
<td>(n = 95)</td>
</tr>
<tr>
<td>First tube</td>
<td>2.6 (2.1–3.0)</td>
</tr>
<tr>
<td>(n = 95)</td>
<td>(n = 95)</td>
</tr>
<tr>
<td>After heparin</td>
<td>2.6 (2.1–3.0)</td>
</tr>
<tr>
<td>(n = 94)</td>
<td>(n = 93)</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>2.7 (2.2–3.3)</td>
</tr>
<tr>
<td>(n = 91)</td>
<td>(n = 93)</td>
</tr>
<tr>
<td>After EDTA</td>
<td>2.7 (2.2–3.3)</td>
</tr>
<tr>
<td>(n = 91)</td>
<td>(n = 93)</td>
</tr>
<tr>
<td>After serum</td>
<td>2.7 (2.2–3.3)</td>
</tr>
<tr>
<td>(n = 91)</td>
<td>(n = 93)</td>
</tr>
</tbody>
</table>

PT, prothrombin time; INR, international normalized ratio (reference range: <1.2); APTT, activated partial thromboplastin time (reference range: 24–31 s); CI, confidence interval; IQR, interquartile range. P-values derived from Wilcoxon signed rank test.
In this study, we evaluated the effect of the ‘order of draw’ on the prothrombin time/international normalized ratio (PT/INR) and the activated partial thromboplastin time (APTT) when the citrate tube is drawn as the first tube (without a prior discard tube), second tube, after a heparin tube, after a serum tube with clot activator and after an EDTA tube. The results from the second citrate tube are considered as the reference value.

MATERIALS AND METHODS

The study was conducted in two phases. In each phase, the study population consisted of approximately 100 consecutive outpatients referred to the oral anticoagulant clinic of the Onze-Lieve-Vrouwe hospital in Aalst, Belgium, for the routine monitoring of their oral antivitamin K medication. We anticipated this population to comprise a wide range of clotting values and expected any effect to be more apparent in a sample with prolonged clotting time [9]. Each patient provided written informed consent, before participating in the study.

According to the experimental design, multiple blood tubes were collected using the Sarstedt Safety Monovette System (Sarstedt, Nümbrecht, Germany). In phase 1, the following tubes were collected in this order, respectively: citrate – citrate (reference) – lithium heparin – citrate, and in phase 2: serum with clot activator – citrate – citrate (reference) – K$_3$-EDTA – citrate. Tube specifications are listed in Table 1.

The venepunctures were performed by experienced phlebotomists and all tubes were filled up to the nominal volume. The collected tubes were transported to the laboratory within 15 min after collection and centrifuged at 1500 $g$ for 10 min at room temperature. Following separation, the PT/INR and APTT were measured on all citrate tubes using a Siemens BCS XP coagulation analyser, using Innovin as the PT reagent and Actin FSL as the aPTT reagent (Siemens Healthcare, Brussels, Belgium). The results of the analysis were expressed as median and interquartile range. The size and significance of the bias between the results of the different tubes and the reference tube in both experiments were determined with Wilcoxon signed rank tests and Bland–Altman plots using MedCalc for Windows version 11.6 (MedCalc Software, Ostend, Belgium). A $P$-value <0.05 was considered statistically significant.

RESULTS

The results are presented in Table 2 and Figure 1. In summary, there is no significant difference in INR when the PT is measured on the first tube, second tube (= reference) or when the citrate tube is filled after a heparin, EDTA or serum tube with clot activator. The same is true for the APTT when measured on a citrate tube filled after a heparin tube. In contrast however, there is a statistically significant bias between the APTT as measured on the reference tube, compared to when measured on the first citrate tube or a citrate tube filled after an EDTA or serum tube.

DISCUSSION

The results presented above show that there is no statistically significant difference between results of PT/INR when measured on the first citrate tube, after a discard tube, after a lithium heparin, after an EDTA or after a serum tube with clot activator. The same is true for APTT measurements on a citrate tube filled after a heparin tube. There is, however, a small but statistically significant bias present between APTT measurements on the first tube, after EDTA and after a serum tube, as compared to the reference tube. The size of the bias, however, is clinically negligible and would not have led to any different clinical action. Of note, the APTT is longer when measured on the first tube or after a serum tube with clot activator. This is the opposite of what would be expected from cross-contamination with tissue thromboplastin or clot activator. Reversely, the APTT is shorter when measured on a tube filled after an EDTA tube, which again is not what would be expected from EDTA contamination.

Our results regarding the use of a discard tube are in agreement with previous studies [10–13] that have evaluated the necessity of a discard tube for the analysis of PT and APTT. These concluded that a discard tube was not necessary and these findings have led to a revision of the CLSI document H3 ‘Procedures for the collection of blood specimen by venipuncture’ [3]. Three more recent studies by Serin et al. [14], Rajmakers et al. [15] and Smock et al. [16] concluded the same for specialized coagulation testing. Interestingly, Rajmakers et al. [15] and Gottfried et al. [10] also found a slight (0.5s) prolongation of the APTT
Figure 1. Bland–Altman plots for the investigated parameters and tubes. Solid lines indicate mean bias; dotted lines zero bias; hatched lines 95% limits of agreement. APTT, activated partial thromboplastin time; INR, international normalized ratio; ref, reference.
when measured on the first tube. Although the mean bias they found was larger than the bias we found (0.14 s), they also concluded that this was not clinically relevant. These findings suggest that it should be considered to eliminate the discard tube for all coagulation testing in future updates of the CLSI document, except when a winged blood collection set is used.

To our knowledge, our study is the first to investigate the potential cross-contamination of citrate tubes with other anticoagulants or clot activator when the order of draw is not respected. In our study, we found no clinically significant influence on the routine coagulation parameters. This is in line with other studies which investigated the effect of the ‘order of draw’ on a selected number of biochemistry results. In 1996, Majid et al. [17] found no influence on calcium or potassium measurements when the order of draw was not respected using Becton Dickinson Vacutainer® tubes. In 2011, Sulaiman et al. [18] detected no potassium-EDTA contamination of blood samples using an incorrect order of draw using the Sarstedt Safety Monovette System (Sarstedt AG & Co, Nürnberg, Germany). Similar results were obtained in 2013 by Salvaggio et al. [19] using the Terumo Venosafe blood collection devices.

Although we should be careful to extrapolate our results obtained within a well-defined study population, they are strengthened by the conclusions from other studies [17–19] that have found no carryover of additives (e.g. EDTA) between different blood tubes using different blood collection systems and study populations.

These findings continue to undermine the importance of the order of draw. Since the first reports by Sun et al. in 1977 [20] and Calam et al. in 1982 [7], the blood collection systems have evolved significantly. Our results add to the growing evidence that nowadays, the order of draw for modern vacuum tube collection systems is indeed much ado about nothing.

ACKNOWLEDGEMENTS

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17. Majid A, Heaney DC, Padmanabhan N, Spooner R. The order of draw of blood specimens into additive containing tubes not affect potassium and calcium mea-
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