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Short Communication

Order of draw practices in venous blood sampling at clinical biochemistry departments in the Danish health care system

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Keywords: venous blood sampling, order of blood draw, phlebotomy, preanalytical phase, preanalytical quality, inter-laboratory variation

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Abstract
Background
Deviation in blood collection procedures is a central source of preanalytical variation affecting overall analytical and diagnostic precision. The order of draw of venous sampling is suspected to affect analytical results, in particular for coagulation analysis. Here we compare the procedures in venous blood sampling among clinical biochemistry departments to assess the uniformity of order of blood draw and adherence to international guidelines in the Danish health care system.

Methods
We collected venous order of draw procedures from 49 clinical biochemistry departments at 22 public hospitals in Denmark. Procedures were compared to the international guidelines from the Clinical Laboratory Standards Institute (CLSI) and World Health Organization (WHO), and assessed in relation to department ISO 15189:2012 accreditation.

Results
We observed seven different order of draw procedures related to citrate, serum, heparin, and EDTA tubes, and the use of discard tubes in relation to coagulation assays. 31 departments (63.3 %) were found to adhere to CLSI and WHO guidelines. A majority of departments instructs the use of discard tubes before collection for coagulation assays in citrate tubes (44 departments; 89.8 %). The citrate tube was the first sample tube to be drawn for most departments (35 departments; 75.5 %); and the preferred order of non-citrate tubes was serum-heparin-EDTA (36 departments; 73.5 %). Adherence to the CLSI and WHO guidelines was not associated with department ISO 15189:2012 accreditation (p = 0.57).

Conclusions
Venous order of draw procedures are diverse at Danish clinical biochemistry departments and show moderate adherence to international guidelines.
1. Introduction
The majority of errors (46.0%-68.2%) in the total process of laboratory medicine occur in the preanalytical phase (1-4). An important step in this phase is venous blood sampling where the order of draw has been advocated as a potential source of errors. A recent systematic review of the available literature by the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working group for Preanalytical Phase (5) supported the importance of the correct order of draw to prevent contamination due to additive carryover. Evidence-based guidelines have been developed by the Clinical Laboratory Standards Institute (CLSI) (10) and World Health Organization (WHO) (11). However, Danish compliance to international guidelines and inter-laboratory variation is currently not known. Therefore, the aim of this study was to assess the uniformity on venous order of blood draw and adherence to CLSI and WHO guidelines in the Danish health care system. In Denmark, order of draw decisions are not necessarily made at hospital level, and each clinical biochemistry departments follow their own local procedures on venous blood draw. Thus, the uniformity in procedures was analyzed at department level. Furthermore, we assessed whether departmental accreditation by ISO 15189:2012 (Medical laboratories - Requirements for quality and competence) was associated with adherence to international guidelines.

2. Materials and methods
We systematically collected venous order of draw procedures from clinical biochemistry departments that perform blood sampling in all public hospitals in Denmark. We did not include health centers and psychiatric departments. The collection of procedures was performed in the period from February to April 2017. If a hospital had more than one clinical biochemistry department, then procedures were obtained for each department independent of whether or not the department had different procedures or management. If available, procedures were retrieved from hospital websites, otherwise departments were contacted by mail or phone. We also collected data on whether or not the department had received ISO 15189:2012 accreditation, which specifies requirements for quality and competence in medical laboratories (12).

We recorded and compared the order of draw for citrate, serum, heparin and EDTA tubes, as well as the use of discard tubes in relation to coagulation assays. No distinction was made as to whether or not the procedures recommended the use of specific clot activators or gels in the specific tube types. The collected Danish procedures were compared to the guidelines recommended by CLSI and WHO (10,11).
The association between department ISO 15189:2012 accreditation and the adherence to CLSI and WHO guidelines (10,11) was analyzed by chi² test using MS Excel 2010.
Table 1 Venous order of draw guidelines from the Clinical Laboratory Standards Institute (CLSI) and the World Health Organization (WHO) (10,11) and procedures in clinical biochemistry departments (n=49) from 22 public hospitals in Denmark obtained between February to April 2017; for the order of draw of citrate tubes, serum tubes, heparin tubes and EDTA tubes, and the use of discard tubes related to coagulation tubes.

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>Use of discard tube</th>
<th>Recommended order of blood draw</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI (10), WHO (11)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>When coagulation tubes are collected as the first or the only tube</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Using a straight needle: Not for INR and PT</td>
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<tr>
<td></td>
<td></td>
<td>Using a winged blood collection set: Always</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Departments (%) of total, n=49</th>
<th>Achieved department accreditation of ISO 15189:2012 (%) of total</th>
<th>Use of discard tube</th>
<th>1st tube</th>
<th>2nd tube</th>
<th>3rd tube</th>
<th>4th tube</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Departments grouped by procedure</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Group 1</td>
<td>31 (63.3)</td>
<td>23 (74.2)</td>
<td>Citrate</td>
<td>Serum</td>
<td>Heparin</td>
<td>EDTA</td>
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<tr>
<td>Group 2</td>
<td>6 (12.2)</td>
<td>3 (50.0)</td>
<td>Always, if coagulation tubes are collected</td>
<td>Citrate</td>
<td>Serum</td>
<td>Heparin</td>
<td>EDTA</td>
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<tr>
<td>Group 3</td>
<td>4 (8.2)</td>
<td>4 (100.0)</td>
<td>Not specified</td>
<td>Heparin</td>
<td>Citrate</td>
<td>EDTA</td>
<td>Serum</td>
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</tr>
<tr>
<td>Group 4</td>
<td>3 (6.1)</td>
<td>0 (0.0)</td>
<td>Always</td>
<td>Citrate</td>
<td>Heparin</td>
<td>Serum</td>
<td>EDTA</td>
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</tr>
<tr>
<td>Group 5</td>
<td>2 (4.1)</td>
<td>2 (100.0)</td>
<td>Always</td>
<td>Citrate</td>
<td>Serum</td>
<td>Heparin</td>
<td>EDTA</td>
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<tr>
<td>Group 6</td>
<td>2 (4.1)</td>
<td>2 (100.0)</td>
<td>Always, if coagulation tubes are collected (except for</td>
<td>Serum</td>
<td>Citrate</td>
<td>Heparin</td>
<td>EDTA</td>
</tr>
</tbody>
</table>
3. Results

Procedures collected
Venous blood order of draw procedures were collected from 49 clinical biochemistry departments at 22 public hospitals within the Danish public health care system.

Order of draw
We observed seven different variations of order of draw procedures related to citrate, serum, heparin, and EDTA tubes, and the use of discard tubes related to coagulation assays (Table 1). The most frequent order of draw procedure recovering 31 departments (63.3%) were in line with the CLSI and WHO guidelines. However, the remaining 18 departments (12.2%) were found to have diverse procedures with six different order of draw policies.

43 of 49 departments (87.8%) gave procedures for order of draw with 37 (75.5%), 4 (8.2%), and 2 (4.1%) departments recommending the first draw to be citrate, heparin, or serum tube containers, respectively (Figure 1A). When addressing the order of draw for non-citrate tubes, the most frequent order was serum-heparin-EDTA for 36 departments (73.5%), followed by heparin-serum-EDTA and heparin-EDTA-serum for 4 (8.16%) and 3 (6.12%) departments, respectively.

Use of discard tube
Procedures from 39 departments (79.6%) specifically stated that discard tubes should be used before drawing citrate tubes; however, two of these departments (4.1%) specifically excluded discard tubes when INR and PT assays were ordered (Table 1, and Figure 1B). The remaining procedures recommended to always (5 departments; 10.2%), to never (1 departments; 2.0%), or did not specify (4 departments; 8.2%) the use of a discard tube. In total, the procedures from 44 departments (89.8%) de facto recommended the use of a discard tube before drawing a citrate tube.

Department accreditation
In total 35 departments (71.4%) were accredited by ISO 15189:2012 (Table 1). Of the accredited departments 23 out of 31 (74.2%) had procedures in line with the CLSI (10) and WHO guidelines (11). Within the group of departments with diverse procedures 12 of 18 (67.0%) were accredited.

We found no association between department accreditation and adherence to the international CLSI and WHO guidelines (p = 0.57, chi²-test).

4. Discussion
We observed seven different variations of order of blood draw procedures from the clinical biochemistry departments queried. Almost two thirds of the departments were compliant to the guidelines from the CLSI and WHO (10,11).
The translation of evidence-based guidelines of order of draw to clinical practice seems manageable and straightforward. The order of draw guidelines are easy to follow, and are not more time-consuming compared to an established practice. However, the moderate adherence to international guidelines of order of draw at Danish clinical biochemistry departments was unexpected given the ease of following. Considerable factors might be influencing the adoption in practice such as the management of department’s unawareness of the guidelines, old traditions, insufficient time and work pressure. Another reasonable explanation is the insufficient evidence of the outcome not adopting international guidelines. The order of draw is a potential preanalytical contributor to diagnostic errors in the total testing phase, and case studies (8,9) indicate that some test errors goes undetected (13). However, increased evidence about the preanalytical errors and subsequently patient-related consequences might promote better compliance to international guidelines regarding the order of draw (14). It is important to underline that we only report what is described in the procedures collected from the departments. Some steps in the order of draw process may be perceptive assumed by staff within a department and thereby not described explicitly, such as the case of group 2 in the procedures. Furthermore, we cannot be sure that the procedures on the hospitals web sides are up to date.

CLSI (10) recommends using a discard tube to be prime the tubing drawn when using a winged needleblood collection set, thereby ensuring a proper anticoagulant to blood ratio. Several studies (15-20) have shown that PT/INR, APTT and some specialized coagulation assay results are not affected if tested on the first tube collected, without the use of a discard tube. These results dispel the misperception that a discard tube should be collected to avoid the effect of thromboplastin. The discard tube is used to ensure maintenance of the proper ratio in the first tube, as prior studies does have conflicting conclusions due to the impact of under filling on coagulation assay results (15-16, 21). The use of a discard tube is costly, and it would be interesting if future studies could evaluate the bold suggestions not using a discard tube.

Drawing blood is regularly performed outside the laboratory for instance at bedside, at surgical procedures, or may be collected and sent from the local general practitioner. Outside the laboratories, personnel with different professional backgrounds often carry out phlebotomy. These different settings with potential discrepancy in procedures will add variation and increase the risk of errors. It is even suggested that professional phlebotomists are only compliant to the local guidelines in 8.1% of all venous blood collections (12). To ensure the preanalytical quality in clinical laboratory practice it is crucial that every step in the total testing process is performed using evidence-based procedures. National and international harmonization may improve preanalytical
variation in inter-hospital clinical studies and practice. It is an eye-opener to identify this moderate adherence to evidence-based guidelines and such diversity within a small country like Denmark. Also, the accreditation standards does not seem to emphasize the need for standardized guideline-based order of draw procedures, seem there is no consistency in which laboratories achieved accreditation with in regards to neither order of draw nor the use of discard tube. Implementation of correct and standardized procedures inside and outside clinical biochemistry departments will presumably reduce the risk of test result errors, and furthermore increase the comparably of test results from one facility to another. Therefore, we recommend that higher compliance to international guidelines concerning the order of draw should be prioritized in the future. Hopefully, the upcoming recommendation from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working group for Preanalytical Phase (WG-PRE) (22) will encourage professionals throughout Europe to implement evidence-based guidelines of blood collection practices.

**Author contributions**

Katja Kemp Jacobsen and Jeppe Madura Larsen conceived and designed the study, analyzed data, and wrote the manuscript. Ida Brandt, Anne Vindahl Christensen, Bjørk Anine Rimsø, Camilla Julie Krøier and Michelle Sørensen collected procedures and compiled order-of-draw data. Julie Smith and Kathrine Overgaard Foss Jensen provided important intellectual contributions to the study and writing of manuscript.

**Acknowledgements**

The authors would like to thank medical laboratory scientist students, Mathias Bech-Thomasen, Mahamed Y. Hassan, Mona Al-Rashedi, and Tiba Younes for assisting in the collection of procedures.
References

Figure 1a-b The distribution of procedures of order of draw according to the use of a first draw and use of a discard tube. Achieved from 49 clinical biochemistry departments, covering 22 public hospitals in Denmark.

**Figure 1a**

First tube drawn

- No order specified (6) 12%
- Serum (2) 4%
- Heparin (4) 8%
- Citrate (37) 76%

**Figure 1b**

Use of discard tube

- Not specified 8%
- Never 2%
- Always, if coagulation tubes are collected 4%
- Always 10%
- Always, if coagulation tubes are collected 76%
Highlights

- We observed seven different variations of order of blood draw procedures from the clinical biochemistry departments in Denmark.
- Only two thirds of the procedures were compliant to the evidence-based guidelines from the Clinical Laboratory Standards Institute (CLSI) and the World Health Organization (WHO).
- Evidence-based and standardized order of blood draw procedures at all clinical biochemistry departments will presumably reduce the risk of test result errors, and furthermore increase the comparability of test results from one facility to another.
- We recommend that higher compliance to evidence-based guidelines concerning the order of draw should be prioritized in the future.