Effects of Time Delay in Processing Common Clinical Biochemical Parameters in an Accredited Laboratory

Omar J\textsuperscript{a,b}, Wan Azman WN\textsuperscript{a,b}, Tan SK\textsuperscript{a,b}, Abdul Wahab NA\textsuperscript{a,b}, Xin-Yuin S\textsuperscript{c}, Law XL\textsuperscript{a}, Chew HJ\textsuperscript{a}, Husin A\textsuperscript{a,b}, Hans Van Rostenberghe\textsuperscript{a,b}, Abdullah MR\textsuperscript{a}

\textsuperscript{a}School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
\textsuperscript{b}Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
\textsuperscript{c}Hospital Taiping, Jalan Taming Sari, 34000 Taiping, Perak, Malaysia

\section*{ABSTRACT}

\textbf{INTRODUCTION:} Analyte stability time is challenging to employ in clinical settings. The diversity of processing time may affect the accuracy of results. This study evaluates the maximum time delay permissible before sample centrifugation and analysis. \textbf{MATERIALS AND METHODS:} We evaluated 15 serum electrolytes in 40 samples centrifuged and analysed using an automated analyser. The time was divided into 1, 2, 6, and 24 hours. \textbf{RESULTS:} Most analytes studied remained stable for up to 24 hours before centrifugation. However, delayed processing affected the values of potassium, magnesium, LDH, and calcium after 2 hours up to a maximum of 6 hours at room temperature for total protein, phosphate, and sodium. \textbf{CONCLUSIONS:} Most analytes were unaffected by a delay in centrifugation at room temperatures, but several critical analytes were severely affected.

\section*{INTRODUCTION}

Patient care and diagnosis have long relied heavily on the clinical laboratory.\textsuperscript{1} Laboratory testing consists of three stages: pre-analytical, analytical, and post-analytical. While the majority of errors occur during the pre-analytical phase (46–68.2\%) and the post-analytical phase (18.5–47\%), a sizable portion (4–32\%) occur during the intra-analytical phase of the testing process.\textsuperscript{2} Time processing of samples is a crucial aspect in the pre-analytical phase in the laboratory to enable accurate and precise results.

The stability of the analytes in the samples start from collection until the time the samples are analysed. Stability in clinical biochemistry is defined as the space of time in which it maintains its value within the established limits by storing the samples in which the analytes are analysed under specific conditions.\textsuperscript{3} The storage time and other factors such as temperature, light exposures, and solvent evaporation that increase the analyte’s metabolism or make the original property vanish could influence the sample stability.\textsuperscript{4} Apart from that, the transportation of blood to the laboratory should be carried out with proper positioning, and centrifugation and separation within a two hour timeframe should take place for serum or plasma to maintain the sample stability.\textsuperscript{5} A delay in separation from the red blood cells would modify the analytes’ stability, leading to unreliable results, which leads to inappropriate diagnosis and treatment.\textsuperscript{6} Almost all patients admitted to the wards have biochemistry blood investigations carried out. In our tertiary teaching hospital, the biochemistry laboratory receives about 1,000 samples daily. As part of an accreditation laboratory and fulfilling the requirements of MS ISO 15189, and with policies by WHO\textsuperscript{7} and Clinical and Laboratory Standards Institute (CLSI)\textsuperscript{8} in placed, all samples are to be analysed within 2 hours upon collection.\textsuperscript{9} This policy was noted to be difficult to apply in our routine practice, as the time taken to transport samples to the laboratory manually might take longer than 2 hours. Likewise, there is a wide range of outcomes from previous research which relate to the present topic. Several experimental investigations in the literature evaluate the stability of samples in most laboratories under diverse settings. The renal function...
tests (RFT) and liver function test results (LFT) are the most significant contributors to the number of tests performed in our laboratory. This study aimed at examining the stability of these routine chemistry analytes over time periods of 1, 2, 6, and 24 hours before analysis in room temperature using serum gel separator tubes. The data will determine the biochemical analytes significantly affected by the time delay at room temperature. The results may then be used to guide specimen delivery services at Hospital Universiti Sains Malaysia (USM).

**MATERIALS AND METHODS**

This was a cross-sectional hospital-based study. Blood samples for routine chemistry assays were randomly obtained from 40 patients following guidelines approved by the USM ethical committee (USM/JEPEM/17090412), together with the practice of informed consent. All procedures were conducted following the guidelines of the Helsinki declaration. Blood samples were obtained from adult patients by doctors-in-charge during the morning ward rounds. A total of 8 mls of blood were collected from each subject through the venous puncture into a plastic Vacuette® serum container (BD Vacutainer® serum; BD, Franklin Lakes NJ, USA). Each patient was subjected to only one blood taking in this study, and the sample was divided into four different containers (2 mls each). Each container was labelled according to the delay length of the samples contained within; thus, they were Containers 1, 2, 6, and 24. Container 1 comprised of samples which were allowed to clot at room temperature for 30 minutes, and were then immediately centrifuged at 3,500 rpm for 10 minutes, and analysed. Subsequently, containers 2, 6, and 24 hours were kept on the rack at room temperature (25°C) until the specified time to be centrifuged and analysed. The following biochemical analytes were analysed on Architect C8000 and under MS ISO 15189 standards based on the time specified. The RFT panel consists of sodium (Na+), potassium (K+), urea, creatinine, uric acid, total calcium, phosphorus, and magnesium. On the other hand, the LFT panel consists of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total bilirubin. All results were recorded accordingly. The data were analysed using statistical software SPSS 26.0. Stability is the ability of a sample to maintain the initial measured value within specified limits of a constituent over a period of time under specified storage conditions. The results were expressed as the mean value for each analyte. In the determination of the clinical significance, the mean % difference (% relative bias) was calculated from the baseline sample using the formula:

\[
\text{Percentage relative bias} = \frac{(T_x - T_0)}{T_0} \times 100\%
\]

\(T_0\) : mean value at 1 hour  
\(T_x\) : mean value of measured analyte at various time intervals (2, 6, and 24 hours).

Percentage relative bias for paired groups was then compared with the current analytical quality specifications for the desirable bias formula:

\[
0.250 \times (CV_I^2 + CV_G^2)^{0.5}
\]

The biological variation (CV_I = within-subject biologic variation; CV_G = between-subject biologic variation) was based on the Westgard database, first published in 1999 and updated in 2014 by Ricos et al. Statistically significant changes were determined for each analyte by repeated-measures ANOVA. A p-value less than 0.05 were considered significant. A significant difference between the mean at different time intervals was further examined by post-hoc pairwise comparison.

**RESULTS**

The analysis results for 15 biochemical analytes measured in serum samples under different time frames are shown in Table 1 and Table 2. Among the analytes studied in the RFT panel, urea, creatinine, and uric acid were the most clinically stable tests of up to 24 hours at room temperature. Meanwhile, most LFT test panels were clinically stable at room temperature up to 24 hours, but the LDH was seen as the least stable analyte.
1. Sodium
2. Potassium
3. Magnesium
4. Phosphate
5. Calcium
6. Urea
7. Creatinine
8. Uric acid
9. Total protein
10. Albumin
11. ALT
12. AST
13. ALP
14. Total bilirubin
15. LDH

LDH

Table 5

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Significance mean difference (p-value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1 hr &amp; 6 hrs (0.004), 1 hr &amp; 24 hrs (0.006)</td>
</tr>
<tr>
<td></td>
<td>2 hrs &amp; 6 hrs (0.007), 2 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1 hr &amp; 2 hrs (0.013), 1 hr &amp; 6 hrs (&lt; 0.001), 1 hr &amp; 24 hrs (0.004)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1 hr &amp; 6 hrs (&lt; 0.001), 1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>2 hrs &amp; 6 hrs (0.002), 2 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Calcium</td>
<td>1 hr &amp; 2 hrs (0.019), 1 hr &amp; 6 hrs (&lt; 0.001), 1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Urea</td>
<td>2 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Not significant</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1 hr &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td>Total protein</td>
<td>1 hr &amp; 6 hrs (0.003), 1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>Albumin</td>
<td>1 hr &amp; 2 hrs (0.003), 1 hr &amp; 6 hrs (0.003), 1 hr &amp; 24 hrs (0.003)</td>
</tr>
<tr>
<td>ALT</td>
<td>2 hrs &amp; 6 hrs (0.002), 2 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td>AST</td>
<td>2 hrs &amp; 6 hrs (0.002), 2 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td>ALP</td>
<td>2 hrs &amp; 6 hrs (0.002), 2 hrs &amp; 24 hrs (0.001)</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>1 hr &amp; 6 hrs (&lt; 0.001), 1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td>LDH</td>
<td>1 hr &amp; 2 hrs (&lt; 0.001), 1 hr &amp; 6 hrs (0.003), 1 hr &amp; 24 hrs (&lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>6 hrs &amp; 24 hrs (0.001)</td>
</tr>
</tbody>
</table>

* Paired comparison post hoc analysis
and closed caps. The clinical significance and analyte instability is considered if the relative bias exceeds the desirable bias set by Westgard for the particular analyte.

Using our statistical technique, we found that most of the analytes examined were stable for up to 24 hours before centrifugation. However, the electrolytes potassium, magnesium, and calcium were clinically affected after 2 hours. Similarly, serum LDH showed an elevation after 2 hours with a relative bias of around 14–19% from the initial sample. The risks of prolonged serum-clot interaction have been long understood, and quick serum-clot separation was urged. Cellular activity and transmembrane diffusion can alter the concentration of serum electrolytes during lengthy serum clot contact time, thus causing an efflux of potassium and LDH out of the cells, which causes an elevation of these analytes. Therefore, for these analytes, the current recommendation is 2 hours between sample collection and serum separation.

Moreover, the CLSI recommends the separation of serum samples within 2 hours of most analytes. Calcium also showed a significant difference between 2 hours and later. It is additionally worth mentioning that the analysis of Daves et al. analysis showed significant changes from 3 hours and on. Thus, we may conclude that only 2 hours of calcium levels are constant, though the explanation behind this is not clear.

Our results showed that serum total protein, phosphate, and sodium are not stable over a 6 hours period. The total protein and sodium were significantly increased in all samples stored at 25°C. A previous study also found that prolonged serum interaction with cells at room temperature yielded similar results for serum sodium, phosphate, and total protein. The statistical analysis using the repeated measure of ANOVA did not show significant changes in the mean between time intervals for serum total bilirubin and creatinine. Notably, one study has shown significant differences in creatinine levels after 24 hours using the kinetic Jaffe method, whereas the levels were shown to be stable for up to 31 hours using enzymatic creatinine assays. The stated stabilities may vary due to various factors, including variances in storage conditions, tube types methods of analysis, and discrepancies in statistical methods used to determine substantial change.

There is crucial consideration regarding appropriate storage, temperature, and duration for sample analysis following specimen collection. Furthermore, maintaining quality assurance ensures the reliability of technological and instrumental aspects of the laboratory measurements and provides proper storage conditions for samples before the test. In conclusion, the handling of patient specimens should not be taken lightly and should be done in a correct, orderly, prompt, and efficient fashion. Delayed transportation and sample analysis could lead to inaccurate results. Similarly, delaying tests after prolonged serum clot contact would give us incorrect results. We recommend analysing samples within 2 hours from collection to the laboratory, especially for analytes such as potassium, calcium, magnesium, and LDH, to ensure valid results. The present study may also be beneficial in defining acceptable delay durations at room temperature for other analytes when rapid sample collection and processing are not available. Besides this, the recommended methods will minimise pre-analytical error from samples from the stages of drawing to reporting.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

REFERENCES