Effects of Delayed Processing in the Specimens Referred to the Testing Laboratory from the Remote Clinics: Impact Analysis of Cost and Patient Care

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ABSTRACT

Background: Responding to the shortcomings in healthcare funding with an ever increasing demand. The National Health Laboratory Services (NHLS) in South Africa implemented a rationalised consolidation of laboratory services, which resulted in the remote clinics referring specimens to the centralised testing laboratory in the city. Clinics serving chronic disease patients were the most affected due to their high patient numbers, and the impact of test results issued had on medical decisions. This study investigated the impact of logistical delays and also the cost of repeat testing and their effects on patient care.

Methods: A prospective pilot study was performed with thirty-one patients recruited from a local clinic which was 14km away from the referral laboratory with the requests of: serum potassium (K+), Alanine Aminotransferase (ALT), and creatinine (CRT). Three specimens were taken from each patient for the study. The first specimen served as a control (no delay in serum separation). The remaining two samples were intentionally delayed for 24 and 48 hours before separation. All specimens were batched and analysed together to avoid inter-run variation. The results of the control and delayed samples were then compared for their critical differences, i.e. the impact on the patient’s management and the estimation of the cost of repeat testing.

Results: Falsely high levels of serum K+ and CRT as well as falsely low ALT levels were noted in both the 24 and 48 hour delayed samples. Significant increases were seen in 96% of the K+ results, 32.25% of the CRT results at 48 hours. A decrease of ALT levels was seen in 3.3%, which were beyond the reference change values (RCV) of each analyte, which could affect clinical decisions.

Conclusion: The delay in serum separation leads to erroneous results in serum K+, ALT and CRT levels with the following possible consequences:
1) repeat testing or the withdrawal of a fresh sample and retesting;
2) additional tests to confirm the abnormal test;
3) wrong medical decision/s; and
4) a delay in medical decision/s.

The findings revealed the hidden cost incurred by the laboratory, the clinic and the patient, as well as highlighting the impact on overall patient care.

KEYWORDS: serum potassium, serum creatinine, serum ALT, primary health care and NHLS
INTRODUCTION
Laboratory medicine/diagnostics form an integral part of patient management. Shrinking resources restrict the use of laboratory tests for patient management as a strategy for cost containment.1,2 The financial restrictions for both the health sector and laboratory services led to an evaluation to manage cost containment. This evaluation includes consolidation of services for laboratories and demand management for health sectors.2,3 Despite the financial difficulties in the health sector, laboratory test requests have increased by approximately 10% according to Smellie4 and this has been attributed to many factors: including diagnostics to rule out disease, inappropriate use of testing for fear of litigation, clinical experience and confidence in clinical testing by doctors, or availability of information on websites.4 Various strategies are needed to manage demand: these include: multidisciplinary interaction between clinicians and laboratory staff, the use of guidelines, activity and cost utilisation information, electronic gatekeeping to restrict test numbers allowed, and deleting tests from a standard laboratory order form.2,5
With consolidation of laboratories the rationale is to centralise the service provision and improve patient care by integrating key issues where financial, operational, and human resource issues are addressed.6 The laboratory facility that is to handle the centralisation should be fit for purpose operationally in terms of: space, staffing, training, customer service, turnaround time, analysers that are capable of handling the workload, and efficient specimen transportation or referral.7 Any delays in the delivery of samples to these centralised locations, and an increase in workload with a lack of proper staff training, will lead to delayed sample separation and increased turnaround times. This will have a negative impact on some analytes as their stability will be compromised. For example, delayed sample separation will lead to leakage of potassium (K+) out of cells together with alanine aminotransferase (ALT), giving falsely elevated results.8 Creatinine (CRT) levels may also be falsely elevated due to delayed sample separation.4,5
The aim of the study was to evaluate the impact of delayed sample separation on the stability of K+, ALT and CRT. Also to evaluate the cost as well as clinical outcome of patients whose samples were referred from the remote clinics to the consolidated laboratory for routine analysis.

Despite financial difficulties, laboratory test requests have increased by approx. 10%

MATERIAL AND METHODS
Study population and design
The study was a pilot cross-sectional study conducted in the laboratory in collaboration with a particular clinic. The clinic was 14km away from the referring laboratory of the hospital and services a high number of patients with chronic diseases (hypertension and diabetes). The study was approved by the University research and ethics committee. Consent was obtained from all the relevant stakeholders (health district offices, laboratory manager, the clinic managers and lastly the patients). In order to maintain patient confidentiality, names and identities were blinded and only codes were used.
Thirty-one patients were enrolled in the study. The thirty-one patients were monitored for hypertension (8 individuals) and retroviral disease (23 individuals). There were twenty-seven females and four males, with the age range between 21-67 years. For each patient, three venous blood samples were collected in 5mL gel serum separator tubes. The order of collection was sequential, where the first sample was the control (no delay in processing) and the latter two tubes were purposefully delayed for 24 and 48-hour respectively prior to processing. The delayed samples were stored in room temperature ±25°C before being centrifuged. Samples were then centrifuged at 3000 relative centrifugal force for 10 minutes and were stored at -20°C to avoid interrun variation.
After thawing the serum tubes at room temperature, serum potassium (K+), creatinine (CRT) and alanine aminotransferase (ALT) were measured in all three tubes in one run using the Abbott Architect Ci8200 analyser (Table I). The assay’s analytical coefficient of variation (CVa) were: 4.5% and 3.6% for K+ at the medical decision limits [MDL] of 2.5mmol/l and 6.0mmol/l respectively. The CVa for CRT was 2.3% at MDL of 90μmol/l; and for ALT at the MDL of 90U/L the CVa was 2.3%. All tubes were assessed for haemolysis, icterus and lipaemia using standardised serum indices for judging the quality of specimens.

Data analysis
The data was analysed using SPSS version 24 statistical software. Statistical differences were assessed between results from the control tubes, 24-hour, and 48 hour delayed tubes, using simple paired t-test and mean difference plots. A p-value of <0.05 was regarded as statistically significant. Further analysis involved the use of RCV, which determines whether the difference between the control and the delayed sample’s results is due to a change in the disease process or due to a combination of physiological change (known as biological variation) and a variation from the laboratory analysis (analytical variation) (see Table I). Trend

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>RCV</th>
<th>Critical value</th>
<th>Reference ranges</th>
<th>Analytical range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>Kinetic rate method</td>
<td>69%</td>
<td>N/A</td>
<td>&lt;45U/L (males) &lt;35U/L (females)</td>
<td>6-942U/L</td>
</tr>
<tr>
<td>CRT</td>
<td>Modified Jaffe</td>
<td>15.56%</td>
<td>N/A</td>
<td>80-115μmol/l (males) 53-97μmol/l (females)</td>
<td>17.7-3270.8μmol/l</td>
</tr>
<tr>
<td>K</td>
<td>ISE</td>
<td>18.24%</td>
<td>&lt;2mmol/L &gt;6mmol/L</td>
<td>3.5-5.4mmol/l</td>
<td>1-10mmol/l</td>
</tr>
</tbody>
</table>

RCV: reference change value; ISE: ion selective electrode
analysis of mean analyte concentrations over time was also evaluated for the control, 24-hour and 48-hours samples (see Figure 7). The estimated costs for each test was calculated by looking at possible repeat testing by both the laboratory and the clinic for samples above reference range for the clinics or those above or below critical limits for the laboratory (see Table II).

RESULTS

Figures 1, 2 and 3 illustrate the differences observed between the control and the 24 and 48-hour delayed samples. In assessing the significant difference using the paired t-tests, the ALT demonstrated a significant change for both the 24 and 48-hour delayed samples with a p value of 0.00 (p<0.05) (Figure 1). Notably there was no significant difference for the CRT and the K+ at 24 hours with p-values of 0.781 and 0.753 respectively (Figures 2 and 3). However, the 48 hour delayed samples demonstrated a significant difference for both the CRT and the K+ with p-values of 0.00 and 0.01 respectively (Figures 2 and 3).

Figures 4, 5, and 6 show the mean difference plots between the control and delayed samples at both 24 and 48 hours. The mean lines were not at zero due to the skewed distribution of mean differences in the CRT and the ALT levels, whereas with the K+ levels, the mean differences were distributed equally thus a zero line was possible. Looking at the distribution of mean differences, in the serum CRT and K+ there is a proportionate positive bias in the delayed samples as compared to the controls. In comparison the ALT level has a negative bias, but it is not predictable or proportionate to the level of the analyte.

None of the control samples showed haemolysis, but a haemolytic index (HI) of 1 was seen in samples 10 and 17 in the 24 hour and 48 hour delayed samples respectively. An HI index of 1 is about 0.3-1g/L haemoglobin (Hb) as per manufacturers insert. Effects of haemolysis on the ALT cannot be verified due to the decline seen on all samples stored at -20°C. The CRT demonstrated a decreased concentration in the haemolysed samples as compared to the non-haemolysed samples. As would be expected, the K+ demonstrated an increased concentration in both the haemolysed and non-haemolysed samples. None of the samples were lipemic or icteric.

Figure 7 demonstrates a decline in ALT over time while the CRT and the K+ demonstrates increased levels over time. Table II demonstrates the estimated costs due to repeat testing by both the laboratory and the clinic. For laboratories repeat testing is done for results above or below critical limits. For the clinics repeats are done for samples above reference range and most often additional test are done to verify the initial tests especially if the result is discordant with clinical picture. For K+ additional test...
includes other electrolytes (sodium and chloride) and bicarbonate. Whereas, for Creatinine: urea and creatinine clearance are the tests most likely to be added. Lastly for ALT additional tests will include other liver enzymes.

**DISCUSSION**

Correct sample handling determines sample integrity and this plays a critical role in issuing accurate and reliable results. This is one of the essential components of patient care. Literature recommends that specimens sent for routine biochemistry testing should be centrifuged after 30 minutes, but within 2 hours after collection to allow for maximum clotting whilst avoiding haemolysis.\(^\text{10}\)

This study demonstrated that, serum CRT and K\(^+\) were affected by the delay in separation at both 24 and 48-hours, but was more pronounced with the latter when the RCV was used (Figures 2 and 3). The trend analysis in Figure 7 also demonstrates that the CRT and the K\(^+\) were affected by delay in sample separation. Ford \textit{et al} demonstrated that a delay in sample separation caused an increase in CRT from as early as 16 hours. But these were based on percentage change not on the RCV concept. Boyanton \textit{et al} demonstrated that the K\(^+\) was stable for up to 24 hours when stored at ±25°C and markedly increased from 48 hours.\(^\text{11}\)

Contrary to Clarks \textit{et al} study, which demonstrated an increase in the serum ALT with delay sample separation,\(^\text{2}\) our study demonstrated a decline in ALT levels in the delayed samples compared to the control samples. The degree of decline was not significant however, when compared with the RCV (Figure 7).

Regarding the stability of analytes during storage, the ALT level was lower for both the 24 and 48-hour delayed samples as compared to the control. Clark's \textit{et al} also established that the ALT is not influenced if the sample is stored at 4°C before separation. A number of reports in the literature state that the ALT should be analysed on the day of collection as separation as storage of the serum at 20°C will cause a 20% reduction of the ALT concentration, which may explain the decrease in our ALT findings.\(^\text{2}\)

The recommended long term storage for ALT determination is -80°C while room temperature (±25°C) stability has been found to be 3 days and at 2-8°C stability has been found to be 7 days.\(^\text{12}\)

A significant increase in the CRT levels (32%) was observed at 48 hours while there was no increase after a 24 hour delay. Significant increases in the levels of CRT will arise the longer the delay in sample separation, especially from 48-hours. This finding is consistent with studies from Sherperd,\(^\text{4}\) Dirar,\(^\text{9}\) and Marjani,\(^\text{13}\) who attributed this change to formations of pseudo-creatinine substances, which cause interferences with the Jaffe kinetic reaction.\(^\text{4,9,11,13}\) Ford and Berg\(^\text{14}\) demonstrated that a delay in sample separation caused an increase in the CRT from as early as 16 hours. Note, that this was based on the percentage change and not based on the RCV. The overall increase we see with the CRT in the 24 and 48-hours delayed samples are in keeping with the findings of Ford \textit{et al} (2008).\(^\text{14}\) Our study demonstrated a slight decline in the CRT concentration when samples had a haemolytic index (HI) above 1. Lippi demonstrated that haemolysis will overestimate CRT concentrations.\(^\text{15,16}\)

Notably, samples with a HI of zero showed an increase after both 24 and 48-hours delay and this is likely due to the formation of non-specific pseudo-creatine that cause interferences with the Jaffe kinetic reaction.\(^\text{4,9}\)
Figure 4. CRT mean-difference plots (a) between controls and 24, (b) controls and 48 hours

Figure 5. K+ mean-difference plots (a) between controls and 24, (b) between controls and 48 hours

Figure 6. ALT mean-difference plots between controls and 24 (a), controls and 48 hours (b)
As expected, K+ levels were significantly affected by a delay in sample separation.\textsuperscript{17} The increase in both 24 and 48-hour delayed samples, was seen in 8 (26.66\%) and 29 (96.67\%) of the samples with a significant difference as per RCV respectively. Boyanton demonstrated that the K+ was stable up to 24 hours when stored at room temperature (±25°\textdegree C) and markedly increased from 48 hours and this is consistent with our findings. This increase in the K+ was attributed to a failure of the Na-K-ATPase pump as glucose is depleted causing intracellular K+ to move to the extracellular compartment.\textsuperscript{11} The more hours it is delayed, and the more haemolysed the sample is, the higher the K+.

This is in keeping with the literature, where increased contact between cells and serum leads to K+ leaking out of cells.\textsuperscript{10} Table II shows the cost impact, which was determined based on the results outside the reference ranges and above the critical limits. The cost incurred is based on the laboratory repeating the test (in the same sample) or the doctors at the clinic repeating the affected test to verify results (in another sample) or adding an additional test to investigate the first result. The total extra cost incurred was: 7.35\% for the K+, 4.5\% for the CRT and 2.5\% for the ALT carried out by both the laboratory and the clinic. Onyenekwu et al (2014) demonstrated that there was about a 2.9\% increase in laboratory running costs when there was repeat testing of analytes at critical concentrations.\textsuperscript{18} Patients will also spend additional money travelling to and from the clinic for the repeated blood withdrawal, and they may have to miss work for additional visits to the clinic. Of concern is that should the clinician take any decisions based on incorrect results to either: change treatment course, change dosage of same treatment or delay in treatment. Individually or together these can impact negatively on patient care. Limitations of the study include small sample size and batching of the samples to avoid inter-run variation which may have led to a decline in the ALT levels. In routine practice specimens would be analysed for all 3 tests without being batched despite possible delays in their separation.

CONCLUSION

In conclusion, our findings demonstrate that a delay in sample separation leads to falsely elevated CRT and K+ concentrations in serum with conversely falsely low ALT levels due to storage at -20°C. The issuing of erroneous results can compromise patient care and may result in repeat testing or requesting additional tests. The latter two will certainly impact on an already strained health care system here in South Africa.

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REFERENCES