ORIGINAL ARTICLE

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

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ABSTRACT

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. **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. **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. **CONCLUSIONS**: Most analytes were unaffected by a delay in centrifugation at room temperatures, but several critical analytes were severely affected.

INTRODUCTION

Patient care and diagnosis have long relied heavily on the clinical laboratory.[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.[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.[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.[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.[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.[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[7] and Clinical and Laboratory Standards Institute (CLSI)[8] in placed, all samples are to be analysed within 2 hours upon collection.[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 time of 1, 2, 6, or 24 hours. The dedicated delivery person ensured the timely delivery of the samples to the laboratory, and upon arrival, the dedicated technologist managed the samples.

Containers were 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.[4] 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:

$$(Tx - T0) / (T0) \times 100\%$$

T0 : mean value at 1 hour
TX : 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:

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

The biological variation (CV_I = within-subject biologic variation; CV_G = between-subject biologic variation) was based on the Westgard database,[10] 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.

Table 1 Percentage relative bias and mean changes from T0 values to Tx (period) compared to Westgard desirable bias in renal function tests

Analytes (unit)	Mean (SD) T0	2 hrs	6 hrs	24 hrs	% Relative bias 2 hrs	6 hrs	24 hrs	Acceptable Delay Westgard Desirable	Acceptable hrs
Sodium (mmol/L)	135.9 (3.48)	135.9 (3.41)	136.2 (3.25)	138.2 (3.31)	0.00	0.22	1.69	0.23	≤ 6 hrs
Potassium (mmol/L)	3.9 (0.51)	3.9 (0.56)	4.0 (0.60)	4.1 (0.67)	0.00	2.56	5.13	1.81	≤ 2 hrs
Magnesium (mmol/L)	0.93 (0.14)	0.95 (0.14)	0.97 (0.15)	0.99 (0.14)	2.15	4.30	6.45	1.80	≤ 2 hrs
Phosphate (mmol/L)	1.35 (0.42)	1.34 (0.42)	1.31 (0.43)	1.66 (0.52)	0.74	2.96	22.96	3.38	≤ 6 hrs
Calcium (mmol/L)	2.07 (0.16)	2.06 (0. 15)	2.11 (0.15)	2.11 (0.16)	0.48	1.93	1.93	0.82	≤ 2 hrs
Urea (mmol/L)	10.3 (10.10)	10.3 (10.03)	10.4 (10.11)	10.6 (10.07)	0.00	0.97	2.91	5.57	≤ 24 hrs
Creatinine (umol/L)	262.3 (330.05)	261.4 (328.75	262.5 (333.41)	263.4 (334.04)	0.34	0.08	0.42	3.96	≤ 24 hrs
Uric Acid (mmol/L)	398.8 (203.3)	399.4 (203.14	398.3 (202.89)	389.2 (196.15)	0.15	0.13	2.41	4.87	≤ 24 hrs

Table 2 Percentage relative bias and mean changes from T0 values to Tx (period) compared to Westgard desirable bias in liver function tests

Analytes (unit)	Mean (SD) T0	2 hrs	6 hrs	24 hrs	% Relative 2 hrs	6 hrs	24 hrs	Acceptable Delay West-gard desirable bias (%)	Acceptable hrs (25°C)
Total protein (g/L)	66.5 (7.85)	66.7 (7.76)	67.4 (7.82)	67.8 (7.96)	0.30	1.35	1.95	1.36	≤ 6 hrs
Albumin (g/L)	37.1 (5.90)	37.5 (5.91)	37.6 (6.00)	37.6 (6.08)	1.08	1.35	1.35	1.43	≤ 24 hrs
ALT (U/L)	42.1 (48.58)	42.5 (48.76)	41.7 (48.62)	41.8 (48.45)	0.95	0.71	0.71	11.48	≤24 hrs
AST (U/L)	32.9 (33.75)	33.3 (33.42)	33.7 (33.87)	34.1 (34.21)	1.22	2.43	3.65	6.54	≤ 24 hrs
ALP (U/L)	104.6 (59.05)	106.5 (58.84)	109.4 (60.29)	111.43 (62.84)	1.82	4.59	6.53	6.72	≤ 24 hrs
Total bilirubin (umol/ L)	10.3 (4.81)	10.4 (4.79)	10.5 (4.85)	10.3 (4.73)	0.97	1.94	0	8.95	≤ 24 hrs
LDH (U/L)	582.6 (429.73)	620.0 (452.57)	661.9 (494.19)	693.0 (426.88)	6.42	13.61	18.95	4.30	≤ 2 hrs

DISCUSSION AND CONCLUSION

The stability of analytes is an essential factor in producing accurate results. It is complex and involves multiple factors such as blood collection tubes, temperature, separation from blood cells, and analysis time. Numerous studies have been carried out to establish the stability of a variety of analytes,[2,11,12] however, the results of such research varied. The late arrival of samples in our hospital from the ward to the laboratory was unavoidable. Regarding the present study, with the equatorial climate of Malaysia and the need to maintain room temperature accurately, there is a high probability of pre-analytical error. Therefore, the outcomes of this study may act as a guide to avoiding inaccuracy to further test analyses and results.

Table 3 Mean value of serum analytes measured at the different time intervals

	Analytes	Mean (SD) T0	2 hrs	6 hrs	24 hrs	F-stat, df (*p*-value)*
1	Sodium	135.9 (3.48)	135.9 (3.41)	136.2 (3.25)	138.2 (3.31)	32.071, 3 (< 0.001)
2	Potassium	3.9 (0.51)	3.9 (0.55)	4.0 (0.60)	4.1 (0.67)	6.549,1.385 (0.007)
3	Magnesium	0.93 (0.14)	0.95 (0.14)	0.97 (0.15)	0.99 (0.14)	30.970, 3 (< 0.001)
4	Phosphate	1.35 (0.42)	1.34 (0.42)	1.31 (0.43)	1.66 (0.52)	35.592, 1.083 (< 0.001)
5	Calcium	2.07 (0.16)	2.06 (0.15)	2.11 (0.15)	2.11 (0.16)	42.383, 2.122 (< 0.001)
6	Urea	10.3 (10.10)	10.3 (10.03)	10.4 (10.11)	10.6 (10.07)	21.339, 2.186 (< 0.001)
7	Creatinine	262.3 (330.05)	261.4 (328.75)	262.5 (333.41)	263.4 (334.04)	0.738,1.377 (0.435)
8	Uric acid	398.8 (203.30)	399.4 (203.14)	398.3 (202.89)	389.2 (196.14)	23.241, 1.395 (< 0.001)
9	Total protein	66.5 (7.85)	66.7 (7.76)	67.4 (7.82)	67.8 (7.96)	28.127, 3 (< 0.001)
10	Albumin	37.1 (5.90)	37.5 (5.91)	37.6 (6.00)	37.6 (6.08)	5.002, 3 (0.003)
11	ALT	42.1 (48.58)	42.5 (48.76)	41.7 (48.62)	41.8 (48.45)	4.666,2.328 (0.008)
12	AST	32.9 (33.75)	33.3 (33.42)	33.7 (33.87)	34.1 (34.21)	6.637, 3 (< 0.001)
13	ALP	104.6 (59.05)	106.5 (58.84)	109.4 (60.29)	111.4 (62.84)	9.664, 1.896 (< 0.001)
14	Total bilirubin	10.3 (4.81)	10.4 (4.79)	10.5 (4.85)	10.3 (4.73)	0.526, 2.039 (0.596)
15	LDH	582.6 (429.73)	620.0 (452.47)	661.9 (494.19)	693.0 (426.88)	18.813, 2.167 (< 0.001)

*Repeated Measures ANOVA. Sphericity assumed values are used if Mauchly's Test for sphericity is not statistically significant (p>0.05). If sphericity is violated, Greenhouse-Geisser correction values are used if the Epsilon value of Greenhouse-Geisser is <0.75. Otherwise, Hyunh-Feldt correction values are used.

This study examined the stability of routine chemical analytes (renal function tests and liver function test results) over 1, 2, 6, and 24 hours before analysis at the temperature of 25°C using serum plain gel separator tubes

Table 4 Significant mean difference of measured serum analytes at 1, 2, 6, and 24 hours time intervals

	Analytes	Significance mean difference (*p*-value)*
1	Sodium	1 hr & 24 hrs (< 0.001) 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (< 0.001)
2	Potassium	1 hr & 6 hrs (0.004), 1 hr & 24 hrs (0.006) 2 hrs & 6 hrs (0.007), 2 hrs & 24 hrs (0.015)
3	Magnesium	1 hr & 2 hrs (0.013), 1 hr & 6 hrs (< 0.001), 1 hr & 24 hrs (< 0.001) 2 hrs & 6 hrs (0.011), 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (< 0.001)
4	Phosphate	1 hr & 6 hrs (0.001), 1 hr & 24 hrs (< 0.001) 2 hrs & 6 hrs (0.001), 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (< 0.001)
5	Calcium	1 hr & 2 hrs (0.019), 1 hr & 6 hr (< 0.001), 1 hr & 24 hrs (< 0.001) 2 hrs & 6 hrs (< 0.001), 2 hrs & 24 hrs (< 0.001)
6	Urea	1 hr & 6 hrs (0.006), 1 hr & 24 hrs (0.001) 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (0.001)
7	Creatinine	Not significant
8	Uric acid	1 hr & 24 hrs (< 0.001) 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (< 0.001)
9	Total protein	1 hr & 6 hrs (< 0.001), 1 hr & 24 hrs (< 0.001) 2 hrs & 6 hrs (< 0.001), 2 hrs & 24 hrs (< 0.001) 6 hrs & 24 hrs (0.009)
10	Albumin	1 hr & 2 hrs (0.003), 1 hr & 6 hrs (0.001), 1 hr & 24 hrs (0.003)
11	ALT	2 hrs & 6 hrs (0.006), 2 hrs & 24 hrs (0.001)
12	AST	1 hr & 6 hrs (0.008), 1 hr & 24 hrs (< 0.001) 2 hrs & 24 hrs (0.014)
13	ALP	1 hr & 2 hrs (0.023), 1 hr & 6 hrs (0.002), 1 hr & 24 hrs (0.001) 2 hrs & 6 hrs (0.003), 2 hrs & 24 hrs (0.003)
14	Total bilirubin	Not significant
15	LDH	1 hr & 2 hrs (< 0.001), 1 hr & 6 hrs (< 0.001), 1 hr & 24 hrs (< 0.001) 2 hrs & 6 hrs (0.020), 2 hrs & 24 hrs (< 0.001)

* Pairwise 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.[13] 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.[3] 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 25^0C. A previous study also found that prolonged serum interaction with cells at room temperature yielded similar results for serum sodium, phosphate, and total protein.[14]

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 is the levels were shown to be stable for up to 31 hours using enzymatic creatinine assays.[15] 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.[16]

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.

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