# Biochemical Analytes Stability in Refrigerated (2 - 8°c) and Frozen (-20°c) Serum Samples at Kericho County Hospital Laboratory, Kenya

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## ABSTRACT

*Pre-analytical variables, including samples storage, can adversely affect the reliability of medical laboratory results. Add-on tests, alongside unprecedented delays in testing, require that samples are stored in a manner that maintain their integrity and optimally preserve the various analytes. Refrigeration and freezing, are well-documented preservation methods used by medical laboratories for short and long term storage of retained specimen. Different analytes however vary considerably in stability when samples are stored over a period of time. This study aimed at evaluated the stability of selected biochemical analytes in refrigerated (2 – 8°C) and frozen (-20°C) serum, to determine the maximum storage period at which accurate and reliable results can still be obtained at the Kericho County Hospital laboratory, guiding specific analytes delayed and add-on testing storage requirement and timelines. Samples drawn from twenty healthy volunteering adult participants, randomly picked from the local population, were processed and analysed at baseline, then a set of aliquots refrigerated and another frozen, in batches marked for analysis on the 7th, 14th, 21st and 28th days, on a well maintained, accurately calibrated, and quality checked HumaStar 100, an automated biochemistry analyser. Fridge and freezer temperatures were maintained relatively constant at 2 – 8°C and -20°C respectively, with routine monitoring throughout the period. The data was coded and analysed on SPSS version 22. The statistical significance of the difference in the determined analyte concentrations in comparison to baseline levels was evaluated using student paired T-test and Wilcoxon rank test. From the findings, alkaline phosphate, creatinine, and aspartate aminotransferase were found to be stable up to 28 days in the refrigerated and frozen serum, with mean percentage differences less than calculated reference change value of 16.48, 35.97 and 42.25 respectively. Sodium, total and direct bilirubin, were however unstable, as their mean percentage difference was more than the calculated reference change value of 2.16, 0.657 and 1.14. Urea and glucose showed stability at 2-8°C for up to the 14th day, while potassium and chloride were only stable in refrigerated serum up to the 7th day analysis. Freezing improved the stability of all the analytes except bilirubin, ALT and sodium. This study recommends immediate analysis of serum for sodium, bilirubin, and ALT, which should never be part of add-on tests, and freezing of the samples when analysis is delayed for the other analytes.*

**Keywords**: Biochemical Analytes, Freezing, Refrigeration, Serum Samples, Stability

## I. INTRODUCTION

All factors at play across the laboratory testing process can adversely affect sample integrity, accuracy and reliability of the test results (Najat, 2017). This is because the various analytes have varying degree of stability under varying conditions, requiring minimal delay from sample collection to analysis (Ikeda et al., 2020). Errors can occur at any stage of the laboratory testing process adversely affecting the results, which have a direct influence on patient diagnosis, and can affect management and treatment outcomes (Bonini et al., 2002). Pre-analytical errors have been identified to account for more than two-thirds of all the errors registered in the laboratory (Asmelash *et al*., 2020). These not only emanate from patient identification and sample collection processes, but also extend to the post collection handling processes including transport and storage (Alavi *et al*., 2020).

To minimize these challenges, freshly collected samples are preferred for analysis in the laboratory, with a recommendation to separate serum from clotted blood by centrifugation within two hours of collection (Zemlin, 2018).

Even after separation, serum which is the main sample for analysis in the biochemistry laboratory, is subject to degradation and analytes degeneration overtime if analysis is not performed immediately (Hedayati et al., 2020, Zemlin, 2018). The occurrence of factors, including power failure, reagents stock-outs, add-on tests, batch testing, increased workload and other similar developing world challenges, makes delayed testing an inevitable laboratory happening and sometimes very necessary to minimize harm to patients from repeated venepuncture and optimize outcomes (Mrazek *et al*., 2020). Samples storage conditions must therefore be carefully decided and monitored to ensure valid results are obtained when testing is delayed, or additional tests are requested (Flores et al., 2020).

Among the storage techniques applied, refrigeration and freezing are storage methods and conditions, which keep samples at 2 – 8 $^0$C and at -20$^o$C, considerably preserving the analytes and maintaining their concentration for given periods (Gomez-Rioja et al., 2019). However, studies have established that analytes are only stable for a minimum period, and their concentrations change at differing rates even when samples are refrigerated or frozen (Razi et al., 2020). Stability limits of these analytes, at which point the total allowable error is exceeded by their percentage deviation, should therefore be established and verified through rigorous experiments, to guide storage conditions and maximum acceptable periods for delayed and add-on testing (Emre et al., 2021).

The goal of this study was to determine the effectiveness of refrigeration and freezing in the preservation of the listed biochemical analytes in serum, thereby informing on the safe storage periods when add-on or delayed testing can be performed if reliable results must be obtained (Emre et al., 2021). The stability limits from this study will be used as refrigeration and freezing guidelines at this facility and the region, ensuring evidence based timelines, and advising on alternative storage methods when extended delays are foreseen and recollection is hopeless (Widell et *al.*, 2019).

### 1.2 Research Objectives

The main study objective was to evaluate the stability of the biochemical analytes in refrigerated and frozen serum at the Kericho County Hospital laboratory over four weeks' period. The specific objectives included:

i. To determine the concentration changes of the selected biochemical analytes in refrigerated (2 – 8$^o$C) and frozen (-20$^o$C) serum samples at the Kericho County Hospital Laboratory.
ii. To determine the stability limit period for the selected biochemical analytes in refrigerated (2 – 8$^o$C) and frozen (-20$^o$C) serum samples at the Kericho County Hospital Laboratory.

## II. METHODOLOGY

### 2.1 Study Design

This was an experimental study, designed to check changes in the concentration of the selected analytes over a storage period at relatively constant storage temperatures. A dedicated samples storage fridge was used, with temperatures maintained between 2 – 8$^o$C and monitored daily. Freezing also occurred in a dedicated freezer with temperature maintained at -20$^o$C (+/-2$^o$C). No repeat freeze-thawing or fridge replacement happened during the period as samples were divided in sets, and batched for baseline, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day analyses. The samples were analysed on HumaStar 100, which is an automated biochemistry analyser produced, distributed and maintained by "Human Diagnostics Worldwide" company. Routine maintenance was promptly performed on the equipment. Calibration was done, and internal quality control material run and results verified before performing the analysis on study samples. The batch analysis of the refrigerated and frozen samples was done at one week intervals up to the fourth week.

### 2.2 Study Population

The study population were healthy adult residents of Kericho County.

Exclusion Criteria: This study excluded adults who reported use of any therapeutic drug or substance of abuse in the last one month living in the county. Adults with any acute or chronic illness, whether presently on medication or not, were also excluded from the study. Samples presenting with lipemia and haemolysis were also excluded from the study.

### 2.3 Sampling Technique

Volunteer participants were randomly selected from the population. Venepuncture procedure was performed on the consenting participants by a professional phlebotomist, and blood drawn using evacuated tube system, filling four no additive red top vacutainers. Serum was obtained by centrifuging the samples at 3000 rpm for five minutes once clot retraction occurred within two hours of collection. Serum was then transferred into cryogenic vials, divided and labelled according to the analysis plan. The eleven analytes of interest were purposively selected as representatives from the four categories of routinely investigated biochemistry profiles, including enzymes, electrolytes, metabolic substrates, and metabolic wastes.

### 2.4 Data Analysis

The collected data was organized, cleaned, coded and analysed on SPSS version 22. Shapiro-Wilk test for normality was used to assess the distribution of the data. Paired student t test and Wilcoxon rank sum test were used to evaluate the difference between the initial analysis findings and the periodic batch analysis for statistical significance. Potential clinical effect of the change was monitored using reference change value, RCV (Emre et al., 2021). Insignificant errors or change in the analytes concentration was denoted by a mean percentage difference less than the calculated RCV. When the mean percentage change however exceeded the calculated RCV, it implied analyte instability, with potential clinical impact (Gómez-Rioja et al., 2019).

## III. FINDINGS

### 3.1 Stability of Selected Biochemical Analytes in Refrigerated (2 - 8°C) Serum Analysed on the 7th, 14th, 21st and 28th Day

Alkaline phosphate (ALP), showed stability in serum samples stored at 2-8°C up to the 28th day, as percentage mean difference was less than the reference critical value (RCV) of 16.48, indicating no clinical impact. At day 7, the percentage mean difference was 5.22%. By the 14th day it was 11.13%, while by the 21st and 28th days, the mean percentage difference was 12.27% and 11.00% respectively.

Direct bilirubin on the other hand, decreased when serum samples were refrigerated at 2-8°C. Greater percentage mean differences were recorded for this analyte compared to the calculated RCV of 1.14, evidence of clinical impact. At day 7, the percentage mean difference was 8.39%, day 14 was 13%, and day 21 and 28 was 18% and 21%. Total bilirubin similarly decreased in the refrigerated serum specimen, with greater percentage mean difference compared to RCV of 0.675, thus possibility of potential clinical impact. At day 7 percentage mean difference was 1.3%, day 14 was 1.9%, and day 21 and 28 were 2.5% and 2.9% respectively.

Chloride analyte presented stability up to the 7th day, the last point at which the percentage mean difference, -4.25%, remained below the calculated RCV of 4.54. At the 14th, 21st and 28th days testing, the percentage mean differences for chloride analyte were -19.95%, 27.72% and 41.04% respectively, much higher than the calculated RCV of 4.54, suggesting degradation, a potential clinical impact.

Creatinine, a metabolism by-product from creatine phosphate, demonstrated stability in refrigerated serum all through the analysis period, registering percentage mean differences less than the calculated RCV of 35.97. The percentage mean difference at the 7th, 14th, 21st and 28th days were 2.59%, 15.40%, 16.85% and 25.20% respectively, demonstrating a month long stability of creatinine in refrigerated serum without no potential clinical impact.

Glucose on the other hand had stability lasting two weeks in refrigerated serum. The percentage mean differences recorded were 3.26% and 12.39% on the 7th and 14th day respectively. These two were less than the calculated RCV of 13.14 suggesting no potential clinical impact. On the 21st and 28th days analyses however, 19.78% and 17.7%, both of which are above the calculated RCV, reflecting analyte deterioration and potential clinical impact.

Sodium analysis in the serum sample stored 2-8°C had a mean percentage difference of 3.22%, 28.27%, 30.51% and 45.84% for days 7, 14, 21 and 28 respectively. All the scores surpassed the calculate RCV for sodium of 2.16, suggesting instability and potential adverse clinical impact even before the lapse of the first week. Potassium however was stable only up to 7 days as the mean percentage difference of 3.04% was less than the calculated RCV of 10.89. By the 14th, 21st and 28th day analyses, the mean percentage differences were 29.93%, 34.42% and 45.67%, all above the RCV of 10.89, hence posing potential clinical impact to the results.

Blood urea nitrogen analysis showed stability up to 14 days in the refrigerated (2-8°C) serum. The mean percentage difference at this point was 27.96%, which is less than the calculated RCV of 32.15. The analysis on the 21st and 28th registered higher mean percentage difference of 38.60% and 55.62% which was quite way above the calculated RCV of 32.15.

Alanine aminotransferase, ALT, evidenced instability from 21 and 28 days of storage as their median percentage difference was -18.32% and 19.07% compared to calculated RCV of 16.48. AST on the other hand, showed stability when serum samples were kept at 2-8°C for 28 days as the median percentage difference at days 7, 14, 21 and 28 were 4.72%, 13.96%, 9.62% and 17.36% respectively, all of which were less than the calculated RCV of 42.25.

**Table 1**

*Stability of Selected Biochemical Analytes in Serum Samples Refrigerated at 2 – 8ºC over a Period of Time*

| Analyte (Units) | Period in days | Mean | SD | Mean % difference | P value | RCV | Clinical Impact |
|---|---|---|---|---|---|---|---|
| Alkaline Phosphate U/L | Day 1 | 98.60 | 31.118 | | - | 16.48 | No |
| | 7 | 103.75 | 31.850 | 5.22 | 0.000 | | |
| | 14 | 109.58 | 34.750 | 11.13 | 0.000 | | |
| | 21 | 110.70 | 33.592 | 12.27 | 0.000 | | |
| | 28 | 109.45 | 32.295 | 11.00 | 0.000 | | |
| Direct Bilirubin (µmol/L) | Day 1 | 3.93 | 1.107 | - | - | 1.14 | Yes |
| | 7 | 3.60 | 1.122 | -8.39 | 0.000 | | |
| | 14 | 3.42 | 1.136 | -13 | 0.476 | | |
| | 21 | 3.21 | 1.108 | -18 | 0.970 | | |
| | 28 | 3.10 | 1.127 | -21 | 0.012 | | |
| Total Bilirubin (µmol/L) | Day 1 | 10.31 | 4.497 | - | - | 0.675 | Yes |
| | 7 | 10.18 | 4.376 | -1.3 | 0.288 | | |
| | 14 | 10.11 | 4.283 | -1.9 | 0.007 | | |
| | 21 | 10.05 | 4.259 | -2.5 | 0.000 | | |
| | 28 | 10.01 | 4.226 | -2.9 | 0.000 | | |
| Chloride (mmol/L) | Day 1 | 113.02 | 2.829 | - | - | 4.54 | No<br>Yes |
| | 7 | 108.22 | 4.375 | -4.25 | 0.000 | | |
| | 14 | 135.57 | 10.314 | 19.95 | 0.000 | | |
| | 21 | 144.35 | 20.769 | 27.72 | 0.000 | | |
| | 28 | 159.40 | 30.554 | 41.04 | 0.000 | | |
| Creatinine (µmol/L) | Day 1 | 69.45 | 11.34 | - | - | 35.97 | No |
| | 7 | 71.25 | 11.525 | 2.59 | 0.012 | | |
| | 14 | 80.15 | 12.667 | 15.40 | 0.000 | | |
| | 21 | 81.15 | 12.650 | 16.85 | 0.000 | | |
| | 28 | 86.95 | 15.049 | 25.20 | 0.000 | | |
| Glucose (mmol/L) | Day 1 | 4.60 | 0.548 | - | - | 13.14 | No<br>Yes |
| | 7 | 4.75 | 0.564 | 3.26 | 0.002 | | |
| | 14 | 5.17 | 0.543 | 12.39 | 0.000 | | |
| | 21 | 5.51 | 0.615 | 19.78 | 0.000 | | |
| | 28 | 5.39 | 0.901 | 17.17 | 0.000 | | |
| Potassium (mmol/L) | Day 1 | 4.27 | 0.297 | - | - | 10.89 | No<br>Yes |
| | 7 | 4.40 | 0.316 | 3.04 | 0.006 | | |
| | 14 | 5.42 | 0.515 | 29.93 | 0.000 | | |
| | 21 | 5.74 | 0.918 | 34.42 | 0.000 | | |
| | 28 | 6.22 | 1.102 | 45.67 | 0.000 | | |
| Sodium (mmol/L) | Day 1 | 138.30 | 2.408 | | - | 2.16 | Yes |
| | 7 | 142.75 | 5.466 | 3.22 | 0.000 | | |
| | 14 | 177.40 | 11.559 | 28.27 | 0.000 | | |
| | 21 | 180.50 | 16.688 | 30.51 | 0.000 | | |
| | 28 | 201.70 | 32.971 | 45.84 | 0.000 | | |
| Urea (mmol/L) | Day 1 | 3.29 | 0.851 | | - | 32.15 | No<br>Yes |
| | 7 | 3.45 | 0.894 | 4.86 | 0.065 | | |
| | 14 | 4.21 | 1.171 | 27.96 | 0.000 | | |
| | 21 | 4.56 | 1.277 | 38.60 | 0.000 | | |
| | 28 | 5.12 | 1.633 | 55.62 | 0.000 | | |

### 3.2 Stability Selected Biochemical Analytes Stored at -20°C (frozen) Condition for Different Days

Serum direct bilirubin was stable only up to the 7$^{th}$ day analysis, as the percentage mean difference of 0.25% was less than the RCV of 1.14. On the subsequent days however, higher mean percentage differences of 3.56%, 2.80% and 5.34%, compared to the calculated RCV of 1.14, and were recorded, on the 14$^{th}$, 21$^{st}$ and 28$^{th}$ day respectively. Total

bilirubin similarly showed very minimal stability, presenting with mean percentage difference higher than the calculated RCV by the 7th day (mean percentage difference, MPD, of 3.59% verses RCV of 0.675).

Chloride analyte was only stable up to 21 days, as the percentage mean difference at days 7, 14 and 21 were -2.80%, 1.73% and 3.19% respectively against a RCV of 4.54. At 28 days the mean percentage difference was -4.95%, which is greater than the calculated RCV of 4.54 hence posing potential clinical impact. The other electrolyte, potassium, showed stability from 7 to 28 day. The mean percentage difference was 4.96%, 9.60%, 9.41% and 9.13% and the values were less than the calculated RCV of 10.89 thus no possible adverse clinical impact with the results. Sodium however showed instability in serum at -20°C, as the mean percentage difference of 5.50%, 10.77%, 11.42% and 7.38% on the 7th, 14th, 21st and 28th days respectively, were greater than the calculated RCV of 2.16.

Creatinine analyte also remained stable in the -20°C frozen serum until the 28th day analysis. The registered mean percentage difference was less than the calculated RCV (35.97) at day 7, 14, 21 and 28, with values 5.47%, 8.78%, 10.51% and 12.17% respectively, pointing to no potential clinical impact. Glucose similarly showed evidence of stability in -20°C frozen serum up to 28 days as shown by lower mean percentage difference of 4.78%, 6.30%, 2.39% and 1.96% compared to the calculated RCV of 13.14 by the 7th, 14th, 21st and 28th days analyses respectively, hence no evidence of potential clinical impact. Reported results from urea analysis equally confirmed stability of the analyte in frozen, -20°C, serum samples for up to 28 days, recording mean percentage differences less than the RCV of 32.15. The corresponding mean percentage differences were of 5.78%, 9.42%, 12.15% and 12.46% for the 7th, 14th, 21st, and 28th days respectively as shown in table 2.

**Table 2**

*Stability of Serum Samples on Selected Biochemical Analyte Stored at -20°C for Different Days*

| Analyte (Units) | Period (day) | Mean | SD | Mean % difference | P value | RCV | Clinical Impact |
|---|---|---|---|---|---|---|---|
| Direct Bilirubin (µmol/L) | Day 1 | 3.93 | 1.107 | - | - | 1.14 | No |
| | 7 | 3.94 | 1.175 | 0.25 | 0.818 | | |
| | 14 | 4.07 | 1.308 | 3.56 | 0.105 | | Yes |
| | 21 | 4.04 | 1.221 | 2.80 | 0.126 | | |
| | 28 | 4.14 | 1.172 | 5.34 | 0.065 | | |
| Total Bilirubin (µmol/L) | Day 1 | 10.31 | 4.497 | - | - | 0.675 | Yes |
| | 7 | 10.68 | 4.819 | 3.59 | 0.039 | | |
| | 14 | 10.81 | 5.116 | 4.84 | 0.063 | | |
| | 21 | 11.60 | 5.157 | 12.51 | 0.000 | | |
| | 28 | 11.96 | 5.197 | 16.00 | 0.000 | | |
| Chloride (mmol/L) | Day 1 | 113.02 | 2.829 | - | - | 4.54 | No |
| | 7 | 109.86 | 3.266 | -2.80 | 0.000 | | |
| | 14 | 114.98 | 5.348 | 1.73 | 0.125 | | |
| | 21 | 116.63 | 6.645 | 3.19 | 0.018 | | |
| | 28 | 107.42 | 23.270 | -4.95 | 0.265 | | Yes |
| Creatinine (µmol/L) | Day 1 | 69.45 | 11.34 | - | - | 35.97 | No |
| | 7 | 73.25 | 11.916 | 5.47 | 0.000 | | |
| | 14 | 75.55 | 11.185 | 8.78 | 0.000 | | |
| | 21 | 76.75 | 11.769 | 10.51 | 0.000 | | |
| | 28 | 77.90 | 11.689 | 12.17 | 0.000 | | |
| Glucose (mmol/L) | Day 1 | 4.60 | 0.548 | - | - | 13.14 | No |
| | 7 | 4.82 | 0.594 | 4.78 | 0.000 | | |
| | 14 | 4.89 | 0.596 | 6.30 | 0.000 | | |
| | 21 | 4.71 | 0.564 | 2.39 | 0.013 | | |
| | 28 | 4.69 | 0.566 | 1.96 | 0.068 | | |
| Potassium (mmol/L) | Day 1 | 4.27 | 0.297 | - | - | 10.89 | No |
| | 7 | 4.47 | 0.310 | 4.69 | 0.000 | | |
| | 14 | 4.68 | 0.397 | 9.60 | 0.000 | | |
| | 21 | 4.79 | 0.439 | 9.41 | 0.000 | | |
| | 28 | 4.66 | 0.369 | 9.13 | 0.000 | | |
| Sodium (mmol/L) | Day 1 | 138.30 | 2.408 | | - | 2.16 | Yes |
| | 7 | 145.90 | 3.401 | 5.50 | 0.000 | | |
| | 14 | 153.20 | 4.808 | 10.77 | 0.000 | | |
| | 21 | 154.10 | 5.937 | 11.42 | 0.000 | | |
| | 28 | 148.50 | 5.326 | 7.38 | 0.000 | | |
| | Day 1 | 3.29 | 0.851 | | - | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Urea (mmol/L) | 7 | 3.48 | 0.961 | 5.78 | 0.000 | 32.15 | No |
| | 14 | 3.60 | 0.980 | 9.42 | 0.000 | | |
| | 21 | 3.69 | 0.985 | 12.15 | 0.000 | | |
| | 28 | 3.70 | 1.002 | 12.46 | 0.000 | | |

SD Standard deviation, RCV-Reference Critical value

The stability results for alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase when serum was kept at -20°C are presented in Table 3. Analysis done on the 7th, 14th, 21st and 28th days for alkaline phosphatase, evidenced stability of the analyte all through the period. The mean percentage difference values were 6.25%, 14.13%%, 15.76% and 15.76% for the respective days, which were less than calculated RCV of 42.25.
For alanine amino-transferase, significant decrease of the analyte was noted in the frozen, -20°C, serum samples, as depicted by the mean percentage difference values of -16.51%, -19.06%, -38.84% and -30.23% which were all greater than the calculated RCV of 16.48 from the 7th up to 28th day analyses. Aspartate amino-transferase on the other hand displayed stability in the same frozen serum samples, with the 7th, 14th, 21st and 28th day analyses registering median percentage difference of 1.70%, 4.15%, 3.96% and 3.40%, all of which were less than calculated RCV of 42.25, implying no potential clinical impact.

**Table 3**
*Stability of Enzymatic Analytes in Serum Samples Stored at-20°C (frozen) Condition for Different Days*

| **Analyte (Units)** | **Period (day)** | **Median (IQR)** | **Median %difference** | **P value** | **RCV** | **Clinical Impact** |
|---|---|---|---|---|---|---|
| Alkaline Phosphatase (U/L) | Day 1 | 92.00 (80.25-107.25) | | | 42.25 | No |
| | 7 | 98.00 (84.25-112.25) | 6.52 | 0.004 | | |
| | 14 | 105.00 (89.00-118.00) | 14.13 | 0.000 | | |
| | 21 | 106.50 (91.25-118.00) | 15.76 | 0.000 | | |
| | 28 | 106.50 (91.50-115.75) | 15.76 | 0.001 | | |
| Alanine aminotransferase (U/L) | Day 1 | 21.50 (15.03-28.78) | | | 16.48 | Yes |
| | 7 | 17.95 (11.35-24.73) | -16.51 | 0.000 | | |
| | 14 | 17.40 (10.20-22.33) | -19.06 | 0.001 | | |
| | 21 | 13.15 (9.45-20.58) | -38.84 | 0.000 | | |
| | 28 | 15.00 (9.42-21.10) | -30.23 | 0.002 | | |
| Aspartate aminotransferase (U/L) | Day 1 | 26.50 (22.28-29.38) | | - | 42.25 | No |
| | 7 | 26.95 (22.40-29.88) | 1.70 | 0.004 | | |
| | 14 | 27.60 (22.20-30.10) | 4.15 | 0.000 | | |
| | 21 | 27.55 (23.43-30.80) | 3.96 | 0.000 | | |
| | 28 | 27.40 (23.73-29.83) | 3.40 | 0.001 | | |

NB: IQR Interquartile range, RCV reference change value and p value <0.05

## IV. DISCUSSION

The alkaline phosphatase, ALP, determinations on the serum samples showed an increase in activity across both storage temperature condition (2-8°C and -20°C) from day 1 at 0.00 to 11.00 at day 28 when samples were stored at 2-8°C and from 0.00 at day 1 to 15.76 at day 28 when serum samples were kept at -20°C. This finding is in agreement with results from study Divya and Jayavardhanan (2010), which found an increase in ALP activity of 25.7% on ALP

results when serum samples were stored at 2-8°C. The increased activity may be due to occurrence of several ALP iso-enzymes with differing stability at different temperatures, and different levels of activity.

Direct bilirubin results in the current study showed a decrease of 8.39 to 21% with unreliable results (values with potential clinical impact from the baseline values) obtained for refrigerated serum (2-8°C) when analysis was done within 28 days. These current findings are in agreement with the findings of a previous study done (Flores et al., 2020), which found a decrease of 30 to 71.2% at 15$^{th}$ and 30$^{th}$ day. They also found a decrease of 11.4 to 39.8% when serum samples were frozen at -20°C (Flores et al., 2020). Their finding differed with the increase in values of 3.56 to 5.34% noted in the current study at 14$^{th}$ and 28$^{th}$ days respectively, but generally agree with the instability of direct bilirubin as established in this study.

Total bilirubin results for the current study indicated that refrigerated serum samples had decreases in values from 1.3 to 2.9% at 7$^{th}$ and 28$^{th}$ day. This was in agreement with the findings of a study which indicated a decrease of -5.9 to -21.3% at 30$^{th}$ day among aliquots stored at 2-8°C (Hirigo, 2020). Inconsistent findings were noted with a study, which reported stability of total bilirubin, with slightly increased changes of 0.8% at 7$^{th}$ day, when the current study found a decrease of 1.3% on the same day, when samples were stored at 4°C, and on the 28$^{th}$ day, they found a decrease of 2.8% when serum samples were kept at -4°C (Shimizu and Ichihara 2019). These findings were in agreement with the current finding of -2.9% at the 28$^{th}$ day. This implied that serum separation time, level of haemolysis and other interfering substances may affect the stability of total bilirubin. The current results had shown concentrations decrease on total bilirubin and direct bilirubin when serum samples were kept at 2-8°C. However, this change could be produced by photo degradation of bilirubin since these samples were not kept in the dark (Sofronescu et al., 2012)

For chloride, the results of the study indicated that refrigerated serum could only give reliable values if analysis is done within 7 days, but frozen serum could give reliable values for chloride (values with no potential clinical impact from the baseline values) when analysis is done up to 21 days. This is consistent with findings from a study which established insignificant changes in sodium, chloride and potassium analytes in short term serum storage (Fauziah et al., 2021).

Creatinine results in this study showed an increased value from 2.59 to 25.20% and 5.47 to 12.17% for both refrigerated and frozen serum samples at 7$^{th}$ and 28$^{th}$ days. This is consistent with a study which did not detect any potential clinical impact change in creatinine values in both refrigerated and frozen serum samples as they found an increase in value of 4.1% difference between the baseline results and the results after 30 day of serum storage at -20°C ( Kachhawa *et al*., 2017). Another study found out an increase of 17.2 to 22.0% and 7.49 to 10.3% for creatinine values at 15th and 30th days when serum samples were both kept at 2-8°C and -20°C respectively (Flores *et al.*, 2020). These findings agree with the current results which shown also an increase values for creatinine of 15.40 to 25.20 % and 8.78 to 12.17% when serum samples were kept in both temperatures for the same period. Thus demonstrating that the optimal storage condition is obtained by freezing the biological samples, since it could decrease the activity of some proteolytic enzymes that can alter the structure of the analytes (Chua *et al.*, 2018).

Glucose results of the current study shown an increase value of 3.26 to 17.17% for both serum samples kept in 2-8°C and -20°C from 7$^{th}$ to 28$^{th}$ day of storage period respectively. Refrigerated serum samples can give reliable glucose value with no potential clinical impact only if analysis is done within 14 days, but, if samples are frozen at -20°C, reliable glucose results of 1.96% was achieved within 28 days, similar findings of <10% change and minimal bias, up to 8 weeks of storage, between baseline results and serum samples stored at -20°C have been recorded (Pleus *et al*.**,** 2022). Freezing samples therefore preserves glucose for a longer time and suitable when testing is delayed for a long time.

On the other hand, the results for potassium indicated that refrigerated serum (2-8°C) can give reliable values only if analysis is done within 7 days of blood collection, and notably frozen serum (-20°C) can give reliable potassium values within 28 days. Similar potassium results, <10% percentage difference, with serum samples stored at -20°C and analysed within 28 days, have been recorded (Kachhawa *et al*., 2017). The results of sodium in the current study showed an increase in values of 5.50 to 7.38 % on serum samples kept at -20°C at 7$^{th}$ and 28$^{th}$ day respectively. Similar results for sodium, with an increase value of 1.75% when serum sample were frozen at -20°C by the 30$^{th}$ day have also been reported (Kachhawa *et al.*, 2017). These studies agree on the fact that electrolytes are very unstable, and timely analysis is key. Freezing preserves potassium well, however it has minimal effect in improving the stability of sodium.

The results of the study pointed out that refrigerated serum samples for urea analyte can only give reliable values when analysis is done within 14 days, while frozen serum samples can give reliable urea values if analysis is done up to the 28$^{th}$ day of storage. Serum urea values exhibited an appreciable increase over time which agreed with records from a previous study (Kachhawa *et al*., 2017). Urea instability, however, indicated by a substantial decrease (15.6% on average) in levels, has been reported for samples stored at -20°C (Van Vrancken *et al*., 2012). The variations and inconsistencies can be explained by difference in the method of analysis, yet both point to the overall instability of urea in stored serum. Freezing offer better preservation, but results obtained after 14 days must be treated with caution.

ALT results of the current study showed a decrease values across the storage period from the baseline value when serum samples were stored at -20°C. A decrease value of 30.23 was noted at day 28, similar to change of up to -9.5% at day 30 in a previous study (Kachhawa *et al*., 2017). The ALT values for the samples stored at -20°C registered a decrease of 1.19 on day 5, followed by an increase from 8$^{th}$ to 14$^{th}$ day (+17 to +18%) in a study (Divya and Jayavardhanan 2010). These are not in agreement with the current findings, where ALT values decreased from 7$^{th}$ to 28$^{th}$ day (-16.51 to -30.23). All these findings however points to significant changes in the enzymatic activity of ALT when sample storage is prolonged.

AST results of the current study indicated an increase in values on refrigerated serum samples when kept for 28 days. In the current study AST results was increased by 4.72 on days 7 to 17.36% on day 28 which was in agreement with a previous study, which indicated an increase of 3.8% when serum samples were stored at 2-8°C (Cray *et al*., 2009). In another study, a decrease of -15.3% for AST among serum samples stored for 28 days at 2-8°C was found (Shimizu *et al*., 2021). This was not in agreement with the current finding, of an increased value of 17.36% on samples stored for the same period. The changes confirm the sensitive nature of enzymes to variations in environmental factors, including minor temperature changes within the refrigeration and freezing ranges.

Day to day equipment performance, the nature of the enzyme itself, on-board duration of reagents, and minor alteration in temperature level might be plausible factors for the variation. Therefore, it is not recommended to store specimen at these temperatures for more than one month.

## V. CONCLUSIONS & RECOMMENDATIONS

### 5.1 Conclusions

This study found insignificant changes in the concentration of glucose and urea in refrigerated, 2-8 °C, sample for up to 14 days only, while they both remained stable in frozen (-20 °C) serum up to the 28$^{th}$ day analysis. Both total and direct bilirubin showed instability in both refrigerated and frozen serum, registering results with unacceptable deviation by the 7$^{th}$ day analysis. For electrolytes, serum samples for chloride and potassium frozen at -20°C remained unaffected and viable for analysis up to the 28 day. Sodium however showed instability in both refrigerated and frozen serum with deviations indicating significant clinical impact registered at week one analysis. ALP also showed instability in the refrigeration and freezing storage conditions within two weeks of analysis. ALP, Creatinine, and AST remained stable in the serum samples, and can be stored over the full range of temperatures at 2-8 °C or -20 °C for up to one month.

### 5.2 Recommendation

The study recommends immediate analysis of serum samples for sodium, total and direct bilirubin, and ALT to obtain valid and reliable results. No delay or add-on testing acceptable. Refrigeration at 2-8 °C can be used for short term storage of samples for urea, chloride, potassium, creatinine, glucose, ALP, and AST, while freezing at -20 °C can be used for longer term storage up to one month when delayed testing is inevitable and add-on testing is absolutely necessary.

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