Stability of Common Biochemical Analytes in Serum when Subjected to Changes in Storage Conditions and Temperature

P Kughapriya\textsuperscript{1}, JA Elanchezhian\textsuperscript{2}

\textbf{Abstract}

\textbf{Aim:} The aim of the study was to assess the effect of storage time and temperature on the test results of 8 common biochemical analytes in serum.

\textbf{Materials and methods:} Five mL of blood was collected from apparently healthy 80 volunteers in a clot activator tube without anticoagulant. Serum was separated after allowing the sample to clot for 30 minutes at room temperature. Separated serum was made into four aliquots. The first part analyzed within two hours act as a baseline value, while the other three aliquots were stored at room temperature for four hours, at 2–8°C for four hours and at 2–8°C for 24 hours, respectively.

\textbf{Results:} Baseline serum values of common biochemical analytes analyzed within two hours of sample collection on comparison with the same sample stored at room temperature for four hours showed a significant change in the values of glucose, urea, creatinine, total bilirubin, albumin, and total protein. Also the values of glucose, urea and albumin were significantly changed on comparing the baseline sample with the sample that is stored for four hours at 2–8°C. Finally we compared the baseline values with the sample stored at 2–8°C for 24 hours and found a significant change among the values of glucose, total bilirubin, and albumin. Other analytes were found to be stable.

\textbf{Conclusion:} According to our study, storage of common biochemical analytes in serum like glucose, urea, bilirubin, and albumin is not advisable. Serum creatinine and total protein were stable on refrigeration. Total cholesterol and triglycerides were found to be stable.

\textbf{Clinical significance:} In the context of patient centered approach to the delivery of healthcare services, clinical laboratories play a vital role. Despite the improvements made in the laboratory, errors still occur due to inadequate insight about the importance of sample collection and storage procedures. Errors in sample handling affects test report which diminishes the confidence in healthcare services and damages an institution’s reputation. Significant interdepartmental cooperation is very essential for identification and proper management of preanalytical errors which in turn will help to make valuable medical decision and effective patient care.

\textbf{Keywords:} Accuracy, Aliquot, Analytes, Preanalytical, Precision.

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\textbf{Aim}

To evaluate the stability of eight common biochemical analytes in serum samples due to time delay and changes in temperature.

\textbf{Objectives}

- To evaluate the stability of biochemical analytes in serum when stored for different time duration.
- Biochemical analytes were assayed in serum on subjecting them to different temperature conditions.

\textbf{Introduction}

Physicians and clinicians use laboratory test to aid in the diagnosis, monitoring, prognosis and follow up of diseases as we live in an era of evidence-based medicine. Hence precision and accuracy of a test result are important. Several steps in the procedure include patient preparation, sample collection, processing of samples, analysis, reporting, and interpretation of test results. The sources of errors that affect the accuracy of test results are classified into preanalytical, analytical and postanalytical. Preanalytical errors are the most common errors in the laboratories. Many preanalytical variables like specimen storage time, specimen storing temperature can be monitored and controlled thereby reducing the magnitude of the errors and improving the accuracy of the test result.

In our study, we analyzed eight common biochemical parameters in serum in an attempt to detect quantitative variations when stored for different period and temperature.

\textbf{Materials and methods}

A cross-sectional study was undertaken in the Department of Biochemistry, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamil Nadu, India. Eighty healthy persons visiting master health checkup OPD in our hospital were taken up for the study after getting proper informed consent. The study was approved by the ethical committee of our college.

\textbf{Inclusion Criteria}

Healthy volunteers of age group 25–60 years were taken up for the study.

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\textbf{Source of support:} Nil

\textbf{Conflict of interest:} None

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Exclusion Criteria
Critically ill patients, children, and antenatal mothers are excluded from the study.

Specimen Collection and Processing
Five mL of venous blood was collected from 80 volunteers. Among them, there were 37 females and 43 males. Blood was collected in sterile clot activator tubes. The samples were allowed to clot for 30 minutes at room temperature. Samples were centrifuged at 2000 rpm for 10 minutes. Samples with visible hemolysis were excluded from the study. Serum was separated as early as possible within two hours from sample collection and made into four aliquots and analyzed differently using EM 200 autoanalyzer.

• I aliquot: The separated serum is analyzed within two hours at room temperature (20–25° C) and they act as a baseline value.
• II aliquot: Serum is kept at room temperature (20–25° C) and analyzed after four hours.
• III aliquot: Serum is stored in a refrigerator at 2–8° C and analyzed after four hours.
• IV aliquot: Serum is stored in a refrigerator at 2–8° C analyzed after 24 hours.

Analysis
Serum was analyzed for eight common biochemical parameters like glucose, urea, creatinine, triglycerides, cholesterol, total protein, albumin and total bilirubin from all the four aliquots. The parameters were assayed using XL system packs from Erba diagnostics (Trans Asia Biomedicals Ltd Baddi, HP in collaboration with ERBA Diagnostics Mannheim GmbH, Mallaustr, Mannheim/Germany). Quality control values for the analytes that we studied were within ± 2 SD (standard deviation) of their respective target means.

Glucose Measurement: (GOD-POD Method)
The enzyme glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. The enzyme peroxidase catalyzes the coupling of aminobenzinepyrine with phenol to yield a colored quinoneminine complex and its absorbance was determined at the wavelength of 500 nm.

Urea Measurement: (Urease–GLDH Method)
Urea is hydrolyzed by urease to ammonia and CO₂. In the presence of (GLDH) glutamate dehydrogenase, ammonia combines with–ketoglutarate to form L-glutamate and its absorbance was determined at the wavelength of 340 nm.

Creatinine Measurement: (Modified Jaffe’s)
Creatinine reacts with alkaline picrate to yield an orange-red colored complex whose absorbance was measured at 510 nm.

Triglyceride Measurement: (GPO Method)
Triglycerides are hydrolyzed by lipase to glycerol and fatty acid. Glycerol is phosphorylated by ATP with glycerol kinase to produce glycerol-3-phosphate. Addition of the enzyme glycerol-3-phosphate oxidase results in the formation of dihydroxyacetone phosphate and H₂O₂. H₂O₂ reacts with N-ethyl-1-Nsulfohydroxypropyl–m-toluidine and 4-aminoantipyrine catalyzed by peroxidase to form a colored compound and its absorbance was determined at the wavelength of 546 nm.

Albumin Measurement: (BCG Method)
Serum albumin reacts with bromocresol green (BCG) to form a colored compound and its absorbance was measured at the wavelength of 630 nm.

Total Protein Measurement: (Biuret method)
The coordination complex formed between the cupric ions and the nitrogen of the peptide bond gives a purple color product whose absorbance was measured at 550 nm.

Total Cholesterol Measurement: (Cholesterol Oxidase Method)
Cholesterol ester is converted to cholesterol and fatty acid by the enzyme cholesterol esterase. Addition of the enzyme cholesterol oxidase results in the formation of 4-cholesten-3-one and H₂O₂. A colored compound is obtained on addition the enzyme peroxidase, phenol, and 4-aminoantipyrine whose absorbance was determined at the wavelength of 500 nm.

Total Bilirubin Measurement: (Walter and Gerarde Method)
Bilirubin is coupled with diazotized sulfanilic acid in the presence of ethylene glycol and dimethyl sulfoxide to produce a coloured dye whose absorbance was measured at 546 nm.

Statistical Analysis
A p value was derived by applying student T-test using SPSS version 24. For p < 0.05 is considered as statistically significant.

Results
In comparison of the means of the serum analytes analysed within two hours at room temperature (20–25°C) to that of the sample stored for four hours at room temperature showed a significant change as per Table 1 in the values of glucose (p value < 0.001), urea

<table>
<thead>
<tr>
<th>S.no</th>
<th>Analytes</th>
<th>Sample size</th>
<th>Within 2 hours Mean ± SD</th>
<th>4 hours at RT Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose mg/dL</td>
<td>80</td>
<td>121.65 ± 60.13</td>
<td>111.48 ± 56.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>Urea mg/dL</td>
<td>80</td>
<td>26.93 ± 10.24</td>
<td>25.15 ± 10.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>Creatinine mg/dL</td>
<td>80</td>
<td>1.15 ± 0.55</td>
<td>1.11 ± 0.48</td>
<td>0.005</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol mg/dL</td>
<td>80</td>
<td>191.61 ± 44.72</td>
<td>191.10 ± 43.32</td>
<td>0.941</td>
</tr>
<tr>
<td>5</td>
<td>Triglycerides mg/dL</td>
<td>80</td>
<td>161.74 ± 90.09</td>
<td>161.62 ± 89.59</td>
<td>0.993</td>
</tr>
<tr>
<td>6</td>
<td>Total bilirubin mg/dL</td>
<td>80</td>
<td>0.55 ± 0.44</td>
<td>0.51 ± 0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>T Albumin g/dL</td>
<td>80</td>
<td>3.58 ± 0.19</td>
<td>3.61 ± 0.18</td>
<td>0.002</td>
</tr>
<tr>
<td>8</td>
<td>T Protein g/dL</td>
<td>80</td>
<td>7.26 ± 0.52</td>
<td>7.09 ± 0.53</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 1: Changes in the mean value of serum analytes when stored for four hours at room temperature

Mean with superscript a,b,c,d,e,f show significant change.

p < 0.05 statistically significant
Stability of Common Biochemical Analytes in Serum when Subjected to Changes in Storage Conditions and Temperature

Within 2 hours Mean ± SD

<table>
<thead>
<tr>
<th>S.no</th>
<th>Analytes</th>
<th>Sample size</th>
<th>Within 2 hours Mean ± SD</th>
<th>4 hours at 2–8°C Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose mg/dL</td>
<td>80</td>
<td>121.65 ± 60.13</td>
<td>116.4 ± 57.66</td>
<td>&lt;0.001¹</td>
</tr>
<tr>
<td>2</td>
<td>Urea mg/dL</td>
<td>80</td>
<td>26.93 ± 10.24</td>
<td>25.37 ± 9.80</td>
<td>&lt;0.001²</td>
</tr>
<tr>
<td>3</td>
<td>Creatinine mg/dL</td>
<td>80</td>
<td>1.15 ± 0.55</td>
<td>1.13 ± 0.48</td>
<td>0.082</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol mg/dL</td>
<td>80</td>
<td>191.61 ± 44.72</td>
<td>192.81 ± 43.79</td>
<td>0.268</td>
</tr>
<tr>
<td>5</td>
<td>Triglycerides mg/dL</td>
<td>80</td>
<td>161.74 ± 90.09</td>
<td>160.80 ± 87.78</td>
<td>0.494</td>
</tr>
<tr>
<td>6</td>
<td>Total bilirubin mg/dL</td>
<td>80</td>
<td>0.55 ± 0.44</td>
<td>0.55 ± 0.44</td>
<td>0.987</td>
</tr>
<tr>
<td>7</td>
<td>T Albumin g/dL</td>
<td>80</td>
<td>3.58 ± 0.19</td>
<td>3.63 ± 0.17</td>
<td>0.002²</td>
</tr>
<tr>
<td>8</td>
<td>T Protein g/dL</td>
<td>80</td>
<td>7.26 ± 0.52</td>
<td>7.30 ± 0.63</td>
<td>0.583</td>
</tr>
</tbody>
</table>

¹,²,³ show significant change

* p < 0.05 statistically significant

Discussion

Small changes in specimen processing or handling can have dramatic effects in analytical reliability and reproducibility of test results. Serum is the liquid portion of the blood devoid of cellular elements and clotting factors. Blood samples require 30–60 minutes to clot at room temperature. When the serum samples were allowed less than 30 minutes then, retained cells and contamination can occur. When the serum samples were allowed more than 60 minutes, cellular components may be released due to cell lysis. Ideal time of serum separation from the sample for most of the common biochemical analytes is within two hours as per the recommendations of Clinical and Laboratory Standards Institute. The time of storage of serum after separation and the temperature at which they are stored affect the test results. Improper methods may be followed in the laboratory due to the increase in sample load. Our study aims at identifying the stability of some common analytes when subjected to different storage conditions.

In our study, delay in the analysis showed changes in serum glucose which could be attributed to its utilization by glycolysis. Glucose is required for cellular metabolism and the rate at which glucose is depleted is dependent on temperature and time. At higher temperatures, there is a higher metabolic rate and glucose is depleted quickly, whereas at lower temperatures it is depleted more slowly.¹¹,¹² No preservatives were added in the glucose sample in our study. Sodium fluoride is preferred as a preservative added to blood samples collected for glucose estimation to prevent glycolysis.¹³

We observed a significant change in urea levels as per Table 2 in which the mean urea concentration stored for four hours at 2–8°C (25.37 ± 9.80 mg/dL) was compared to urea analyzed within two hours at room temperature (26.93 ± 10.24 mg/dL). This was in contrast to some study where urea sample was found to be stable for 15 days when stored at 0–4°C.¹⁴ The decrease in urea concentration may not be considered as clinically significant though there is statistical significance. This could be attributed to

Table 2: Changes in the mean value of serum analytes when stored at 2–8°C for 4 hours

<table>
<thead>
<tr>
<th>S.no</th>
<th>Analytes</th>
<th>Sample size</th>
<th>Within 2 hours Mean ± SD</th>
<th>4 hours at 2–8°C Mean ± SD</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Glucose mg/dL</td>
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<td>Urea mg/dL</td>
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<td>25.37 ± 9.80</td>
<td>&lt;0.001²</td>
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<tr>
<td>3</td>
<td>Creatinine mg/dL</td>
<td>80</td>
<td>1.15 ± 0.55</td>
<td>1.13 ± 0.48</td>
<td>0.082</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol mg/dL</td>
<td>80</td>
<td>191.61 ± 44.72</td>
<td>192.81 ± 43.79</td>
<td>0.268</td>
</tr>
<tr>
<td>5</td>
<td>Triglycerides mg/dL</td>
<td>80</td>
<td>161.74 ± 90.09</td>
<td>160.80 ± 87.78</td>
<td>0.494</td>
</tr>
<tr>
<td>6</td>
<td>Total bilirubin mg/dL</td>
<td>80</td>
<td>0.55 ± 0.44</td>
<td>0.55 ± 0.44</td>
<td>0.987</td>
</tr>
<tr>
<td>7</td>
<td>T Albumin g/dL</td>
<td>80</td>
<td>3.58 ± 0.19</td>
<td>3.63 ± 0.17</td>
<td>0.002²</td>
</tr>
<tr>
<td>8</td>
<td>T Protein g/dL</td>
<td>80</td>
<td>7.26 ± 0.52</td>
<td>7.30 ± 0.63</td>
<td>0.583</td>
</tr>
</tbody>
</table>

¹,²,³ show significant change

* p < 0.05 statistically significant

Table 3: Changes in the mean value of serum analytes when stored at 2–8°C for 24 hours

<table>
<thead>
<tr>
<th>S.no</th>
<th>Analytes</th>
<th>Sample size</th>
<th>Within 2 hours Mean ± SD</th>
<th>4 hours at 2–8°C Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose mg/dL</td>
<td>80</td>
<td>121.65 ± 60.13</td>
<td>111.74 ± 59.03</td>
<td>&lt;0.001¹</td>
</tr>
<tr>
<td>2</td>
<td>Urea mg/dL</td>
<td>80</td>
<td>26.93 ± 10.24</td>
<td>26.93 ± 10.65</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>Creatinine mg/dL</td>
<td>80</td>
<td>1.15 ± 0.55</td>
<td>1.13 ± 0.48</td>
<td>0.310</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol mg/dL</td>
<td>80</td>
<td>191.61 ± 44.72</td>
<td>191.54 ± 44.11</td>
<td>0.950</td>
</tr>
<tr>
<td>5</td>
<td>Triglycerides mg/dL</td>
<td>80</td>
<td>161.74 ± 90.09</td>
<td>160.50 ± 90.19</td>
<td>0.411</td>
</tr>
<tr>
<td>6</td>
<td>Total bilirubin mg/dL</td>
<td>80</td>
<td>0.55 ± 0.44</td>
<td>0.51 ± 0.44</td>
<td>&lt;0.001²</td>
</tr>
<tr>
<td>7</td>
<td>T Albumin g/dL</td>
<td>80</td>
<td>3.58 ± 0.19</td>
<td>3.66 ± 0.17</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>8</td>
<td>T Protein g/dL</td>
<td>80</td>
<td>7.26 ± 0.52</td>
<td>7.26 ± 0.79</td>
<td>0.966</td>
</tr>
</tbody>
</table>

¹,²,³ show significant change

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In our study, statistical instability was observed when total protein was stored at room temperature, other samples stored at 2–8˚C were stable. We also observed changes in serum albumin in all the stored samples. The stability of proteins in serum samples are temperature dependent. Steps should be taken to prevent delay in sample processing to minimize the degradation of proteins.

This study helped us to identify the useful length of storage time for common serum analytes.

**Conclusion**

In our study, we observed a significant change among common biochemical analytes in serum like glucose, urea, bilirubin, and albumin upon storage over a different period and temperature. So it is advisable to assay these samples within 2 hours after receiving the samples. Serum creatinine and total protein showed no significant changes in refrigerated samples. Total cholesterol and triglycerides were found to be stable even after some time delay. This study helped us to identify sensitive analytes that significantly vary when not stored properly. The results from our study helped us to determine those analytes that produce valid results despite exposure to variable storage conditions. This knowledge will help us to improve the precision and accuracy of current diagnostic strategies.

**Study Limitation**

- Sample size was small.
- Run to run variation was not studied.
- Analysis was not done in duplicates to ensure the reliability of results.

**References**