Effect of Storage Time and Temperature on some Serum Analytes

Ahmed M Dirar, Daleel A Abdallah and Kamal Eldin A Abdelsalam

Objective: To determine the effects of storage time and temperature on the laboratory results of 10 analytes in sera from apparently healthy adult males.

Study Design: Prospective Analytical Cross Sectional Study

Study Setting: Department of research laboratory, college of higher studies, Open University of Sudan, clinical chemistry, college of medical laboratory sciences Omdurman, Islamic university, Division of chemical pathology, department of medical laboratory sciences, college of health sciences, Omdurman Ahlia University

Place & Duration: Khrdufan states, Sudan, from March 2008 to December 2009.

Materials & Methods: We determined the concentration of Glucose, Urea, Uric Acid, Creatinine, Albumin, Total protein, Total cholesterol, Calcium, Phosphorus and Triglyceride in serum (using laboratory kits and spectrometric technique) collected from 100 apparently healthy adult males. 10ml of venous blood was collected, and the serum was separated after 20 minutes stored for 0, 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hours at each of the following temperatures: In fridge with 4 ± 1°C and in laboratory bath with 23 ± 1°C.

Results: Glucose, phosphorus and uric acid were the least stable and the serum should be determined within 48 hrs at 4±1°C and at 23±1°C for these analytes. The other analytes were stable for 72 hrs. Proper storage temperatures and times must be considered for the glucose, phosphorus and uric acid if delaying can not be avoided after sample collection.

Keywords: Storage time, temperature, serum analytes.

Introduction

Laboratory tests are used by clinicians for diagnosis, monitoring, and prognosis in patients with different diseases. A number of factors, primarily preanalytical and analytical as well as normal biological variations affect the accuracy of test results. Preanalytical factors such as sample collection and handling, diet, exercise and drugs can all affect a test result. The key characteristics of any test are its bias and imprecision. Bias is primarily an analytical characteristic, in which reported results differ from the actual value. Imprecision, or reproducibility error, is due to both physiological and analytical factors. To detect real pathological changes in patients, the preanalytical and analytical variations must be reduced to acceptable levels at which they cause no adverse effect on the results.

Observational clinical studies can often be greatly enhanced by the inclusion of biochemical analyses in stored serum samples collected from the population being studied. Biochemical analyses can be used to assess risk factor exposure, to control for disturbing, or to measure the effects of bias. In randomized trials, biochemical analyses can be used to monitor the safety and biochemical efficacy of treatment. Standard guidelines for blood sample handling state that plasma or serum should be separated (within 20-30 min) from cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analytes. Although this is necessary for particular analytes, it might be assumed that many blood analytes drop within a matter of hours in no separated samples kept at room temperature. For most routine assays in a clinical laboratory, serum is the preferred sample. The laboratory receives the specimen as a whole blood, and then separates the serum from the clot by centrifugation. For clinically useful and reliable test results, the time between blood collection and serum separation must be controlled. Many investigators have studied related changes in some analytes, but the results are controversial. The 10 analytes selected for this study have not been previously studied in this area in healthy subjects. For this reason the present study was designed to determine the effect of storage time and temperature...
on the laboratory results of 10 analytes in sera from apparently healthy adult males in the 3 Kurdufan states. In this study we also tried to find out the quantitative alterations and the useful length of stored serum in different time and temperature on the laboratory results.

Materials and Methods
The subjects were healthy adult males and about 25-50 years old, from employees of Kurdufan University - Faculty of medicine & health sciences. The adult individuals were instructed to fast overnight until blood collection was completed. 10 ml of blood was collected from each male (in total 100 adult males) without the use of an anticoagulant. Samples were allowed to clot at room temperature for 20 min. Samples were then centrifuged and the separated sera were assayed for the selected analytes without delay.

In addition one sample from each participant was stored for 0, 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hours at each of the following temperatures: In refrigerator at 4 ± 1°C and in laboratory bath at 23 ± 1°C.

We determined the concentration of Glucose3, Urea4, Uric Acid5, Creatinine6, Albumin7, Total protein8, Total cholesterol9, Calcium10, Phosphorus11 and Triglyceride12 in serum using laboratory kits and spectrophotometry technique (model JENWAY, IOS UV/VIS) in the laboratory of Biochemistry(Faculty of Medicine). The samples showing visible hemolysis were excluded from the study. Data were analyzed using paired t-test for comparison of different storage time and temperature. Results were considered significant when p value was <0.05.

**Assay analysis procedures:** All the analytes were assayed using standard analytical procedures as previously described (3-12)

**Results**
The effects of storage time and temperature on results for the following assays were not statistically significant: Urea, Uric Acid, Calcium, Total protein, Albumin, Triglyceride and Total cholesterol. Statistically significant changes as compared with the initial time values (table I) were noticed for 3 constituent (table II and III).

Table I: Assay values in sera separated immediately after collection (Zero time) from 10 healthy males (with reference intervals1).

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Average</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>92.3</td>
<td>70 - 110</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>29.7</td>
<td>15 - 45</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8</td>
<td>0.4 - 1.4</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4</td>
<td>2 - 7</td>
</tr>
<tr>
<td>T. Cholesterol (mg/dL)</td>
<td>166.5</td>
<td>120 - 220</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>110</td>
<td>70 - 200</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.6</td>
<td>8.8 - 10</td>
</tr>
<tr>
<td>Phosphorous (mg/dL)</td>
<td>3.3</td>
<td>2.7 - 4.7</td>
</tr>
<tr>
<td>T. Protein (g/dL)</td>
<td>7.3</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.8</td>
<td>3.5 - 5</td>
</tr>
</tbody>
</table>

Table II: Assay values changes in analyte concentration over time in sera stored at 4±1 °C

<table>
<thead>
<tr>
<th>Time</th>
<th>Glucose (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>T. Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorous (mg/dL)</th>
<th>T. Protein (g/dL)</th>
<th>Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>29.7</td>
<td>0.7</td>
<td>4</td>
<td>166.4</td>
<td>110</td>
<td>9.6</td>
<td>3.3</td>
<td>7.3</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>89.5</td>
<td>29</td>
<td>0.7</td>
<td>4</td>
<td>166.4</td>
<td>110</td>
<td>9.6</td>
<td>3.3</td>
<td>7.3</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>89.5</td>
<td>29</td>
<td>0.7</td>
<td>4</td>
<td>166.2</td>
<td>109.8</td>
<td>9.6</td>
<td>3.3</td>
<td>7.2</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>89.1</td>
<td>28.6</td>
<td>0.7</td>
<td>3.9</td>
<td>166.2</td>
<td>109.8</td>
<td>9.6</td>
<td>3.3</td>
<td>7.3</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>87</td>
<td>28.5</td>
<td>0.7</td>
<td>3.8</td>
<td>165.9</td>
<td>109.8</td>
<td>9.6</td>
<td>3.3</td>
<td>7.2</td>
<td>4.8</td>
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<tr>
<td>6</td>
<td>83</td>
<td>28.3</td>
<td>0.5</td>
<td>3.8</td>
<td>165.8</td>
<td>109.7</td>
<td>9.4</td>
<td>3.3</td>
<td>7.1</td>
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<tr>
<td>7</td>
<td>79</td>
<td>28.2</td>
<td>0.5</td>
<td>3.4</td>
<td>165.8</td>
<td>109.7</td>
<td>9.4</td>
<td>3.5</td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>78</td>
<td>28</td>
<td>0.5</td>
<td>3.3</td>
<td>165.8</td>
<td>109.7</td>
<td>9.2</td>
<td>3.5</td>
<td>6.8</td>
<td>4.7</td>
</tr>
<tr>
<td>24</td>
<td>74</td>
<td>27.7</td>
<td>0.5</td>
<td>3</td>
<td>165</td>
<td>109.6</td>
<td>9</td>
<td>3.8</td>
<td>6.9</td>
<td>4.7</td>
</tr>
<tr>
<td>48</td>
<td>71.6</td>
<td>27.4</td>
<td>0.5</td>
<td>3</td>
<td>164.7</td>
<td>109.6</td>
<td>8.9</td>
<td>4</td>
<td>6.7</td>
<td>4.7</td>
</tr>
<tr>
<td>72</td>
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<td>109.5</td>
<td>8.8</td>
<td>4.4 *</td>
<td>6.4 *</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Statistically significant variation (p value < 0.05)
for urea, uric acid, calcium, total protein, albumin, triglyceride and total cholesterol there was no effect on samples stored at 4 ± 1 °C and 23 ± 1 °C for as long as 72 h (table 2 and 3). The suitable tests for analysis at 48 h incubation at 4±1 °C but not suitable for analysis at 48 h incubation at 23±1 °C (suitable for analysis at 24h) were glucose, phosphorus and uric acid (table II and III).

### Discussion

There is a lack of consensus regarding the most appropriate specimen type for analysis of many biochemistry analytes. Information on the stability of serum analytes during storage of serum is often incomplete and sometimes contradictory. In this study, effect of storage at room temperature (23±1°C) and refrigeration (4±1°C) for 0, 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hours on 10 serum analytes were investigated. We found that only glucose, creatinine and phosphorus were affected by storage at 4 ±1 °C and 23 ±1 °C for 48 h and 24 h respectively. Glucose was decreased after 48 h and 24 h at 4 ±1 °C and 23 ±1 °C respectively. Creatinine and phosphorus were increased after 48 h and 24 h at 4±1°C and 23 ±1°C respectively. An important advantage of this study when compared with those already published was the number of different storage times.

Donnelly et al [13] investigated the stability of 25 analytes from serum of healthy donors and stored at room temperature and at 4 °C over 48 h, 14 days and 4 months respectively. All 25 analytes were stable at that temperature for specified times. Study of Saeed et al [14] on camel serum showed that albumin, calcium, Phosphorus and cholesterol did not change over 9 days when stored at 4-5 °C. At 4-5 °C, creatinine and glucose in camel serum remained stable for 6 days, total protein for 7 days and blood urea nitrogen for 8 days. At room temperature (23-25 °C), total protein, albumin, calcium and phosphorus were stable over 72 h. Changes in glucose occurred after 3 days. Bobby et al [15] investigated the stability of 24 analytes after immediate separation of serum and after storage at room temperature (25 °C) and analyzed in 0, 2, 4, 8, 16, 24, 32, 40, 48 and 56 hours after collection. All analytes in serum were stable over 56 h periods. Heins et al [16] determined the effects of storage time and temperature on 22 serum analytes. In serum at 9°C for seven days the mean changes in phosphorus exceeded significantly. While at room temperature, phosphorus, uric acid and triglycerides increased continuously. Our results were in agreement with those reported by Donnelly et al and Saeed et al [13-14] but differed from those of some of the other investigators [15-16]. Changes in the concentration of glucose, phosphorus and uric acid are clinically significant with increasing storage temperature. At temperature 23 ±1 °C the concentration of glucose in serum decreased and the concentration of phosphorus and uric acid in serum increased with increasing temperature when compared with initial results with those obtained after storage of 4°C. The decrease of glucose concentration during storage may be related to sensitivity of glucose to temperature variations. An abnormally high
phosphorus and uric acid concentration could also be the result of prolonged storage of serum at room temperature (23±1 °C). Recording the length of time from collection to separation of each sample might allow appropriate adjustment to be made for the slight increase in concentration of these analytes over time. It should also be noted that room temperature in the present study was 23±1 °C which may not be realistic for studies in hotter climates. Some routine tests can tolerate fairly long delays (72 h) at room temperature without changes in analyte content. Samples for glucose, phosphorus and creatinine should be processed within 24 h and 48 h. The remaining analytes evaluated were stable for 72 hours. Proper storage temperature and times must be considered for these analytes (glucose, phosphates and uric acid) if measurement is not to take place immediately after specimen collection. It is pertinent to mention that besides assuring proper storage condition of samples before assay, appropriate quality assurance measures should be adopted to ensure the reliability of technical and instrumental aspects of the laboratory determinations. In conclusion we hope that the results we have presented will help assess which of the constituents may be assayed in serum stored for prolonged periods under commonly encountered storage conditions when such prolonged storage occurs in advertently or is unavoidable. We recommend that samples should be analyzed in the laboratory within 24 h of collection to ensure valid results. In addition, the turn-around time from sample drawing to reporting the analytical result would be shortened.

References