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# Effect of storage time and temperature on serum clinical biochemistry analytes

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## ABSTRACT

**Objective:** The aim of the present study was to determine the effect of storage time and temperature on laboratory results of routine clinical biochemistry analytes in sera from apparently healthy volunteers. **Materials and methods:** Ten healthy volunteers were instructed to fast overnight and 10 ml of blood was collected from each subject without anticoagulant (in red capped vacutainer). Samples were allowed to clot at room temperature for 20 min, centrifuged and serum separated which was stored in various aliquots. Baseline analysis ("0" day values) of 18 analytes in serum of each subject was done without delay on the same day of collection. Other aliquots were stored at 0° C and 4±1° C with cover of aluminium foil to avoid exposure of direct light and analysed on 3, 7, 15 and 30 days. **Results:** Urea, uric acid, phosphorus, TG and HDL were stable till 7 days whereas ALP was stable till 15 days but SGOT was stable upto 30 days at both 0° C and 4±1°C. ALP, Amylase and urea were stable up to 30 days at 0o C temperature. All analytes showed significant variation on 3rd day which were stored in room temperature except calcium which was stable. But glucose, creatinine, inorganic phosphorus and potassium were least stable and should be determined within 48 hours at 4±1° C and 24 hours at 23±1° C for these analytes. **Conclusion:** Various routine biochemical analytes were stable using the storage conditions tested in this study at least up to 7 days in usual refrigerator. This evidence can be used in exceptional circumstances because processing of any analyte on the same day should be done for better reproducibility. Beyond all this, it is even very important and useful to check the reliability of technical and instrumental resources that the laboratory will use during the study because molecular alterations of the analytes due to variable storage conditions can cause misleading results.


## KEYWORDS

Clinical biochemistry;
Serum analytes;
Storage time and temperature.

## INTRODUCTION

The diagnosis, treatment, and follow-up of patients relies heavily on the accurate measurement of numerous blood, serum, and plasma analytes.1,2 The principal sources of error affecting the accuracy of any laboratory test result can be categorized as preanalytical, analytical, or postanalytical.1-6 In one study2 of the distribution of each of these types of error and their impact on patient outcomes, the frequency of errors in each category decreased in this order: preanalytical (62%), postanalytical (23%), and analytical (15%). Thus, preanalytical variables (eg, specimen-storage time and temperature prior to testing) constituted the most commonly occurring causes of error (imprecision, inaccuracy, or both) in laboratory test results.

Many preanalytical variables (eg, specimen collection and handling) can be controlled and monitored, thus reducing the magnitude of the error, or inaccuracy, in laboratory test results associated with these variables. Most clinical laboratories standardize the preanalytical phase.3,6 However, the magnitude of the inaccuracy associated with the effect of preanalytical variables on laboratory test results can be significant. Preanalytical variables raise a critical question: what magnitude of change in analyte values due to preanalytical variables is likely to be clinically significant (ie, result in incorrect or inappropriate treatment of a patient and poorer patient outcomes)? Because the imprecision and inaccuracy of modern automated chemistry analyzers for all analytes are monitored daily and typically are excellent and stable over time, the random error (RE; due to imprecision) and systematic error (SE; due to inaccuracy) components of the analytical total error (TE = RE + SE) is constant, such that any change in analyte values over time during sample storage is due principally to preanalytical variables (ie, specimen-storage time and temperature). The aim is to reduce these variations to acceptable level for diagnosing diseases in patients. Serum is most commonly used in a clinical laboratory as a routine. Serum separated from clot within 20 minutes as long stay with clot may alter analytes quantitatively.

The present study was designed to evaluate the effect of storage time and temperature on the laboratory results of 18 analytes in sera from apparently healthy subjects in Rohtak. In this study we planned to detect the quantitative variations and the beneficial length of stored serum in different time and temperature which provide appropriate laboratory results.

## METHODS AND MATERIALS

This study was done in May 2014. The subjects were ten apparently healthy volunteers among employers of PGIMS, Rohtak (6 males and 4 females, age range 25-45 years), who gave written informed consent form for participation in the study.

## BLOOD SAMPLING

Blood was collected by using Vacuette® Standard tube holder and Vacuette® 22-gauge, 0.7 × 25 mm multisample needle. Blood specimens were drawn into plastic tubes: BD Vacutainer® Serum (Ref. No. 367812) (Serum Plain) 6 mL.10 mL of fasting venous blood was collected in the morning from each study subject by a single venipuncture to control for any draw order bias by using 2 vacutainers for each subject.

Sera were allowed to clot for 20 min at room temperature and then centrifuged at 3400 x g for 3 min. Visible hemolysed samples are excluded. Immediately following centrifugation, all specimens were analyzed within 30 min by autoanalyser (RANDOX) and combiline for electrolytes to get 0 day (baseline) values. Serum of each volunteer was separated and stored in 100 sterile clean plastic aliquots having cap to close (to avoid evaporation which leads to concentration) i.e. 500µL/each aliquot- 10 aliquots/volunteer. The aliquots were kept to avoid from light exposure by wrapping the sample trays (9 in number) with aluminium foil and stored in respective temperature conditions with batch name as

1. R-0 for 0 day baseline values.
2. R-3 for 3rd day samples stored in room temperature
3. $4^0$-3 for 3rd day samples stored at $4^0 \pm 1$ C
4. $4^0$-7 for 7th day samples stored at $4^0 \pm 1$ C
5. $4^0$-15 for 15th day samples stored at $4^0 \pm 1$ C
6. $4^0$-30 for 30th day samples stored at $4^0 \pm 1$ C

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TABLE 1

| Sr.No | Analyte | Method | Reference Range | Intra Assay CV (%) |
|---|---|---|---|---|
| 1 | Urea | Enzymatic method (urease) | 10 - 50 mg/dL | 22.3 |
| 2 | Creatinine | Spectrophotometry (picricate) | 0.7- 1.3 mg/dL | 9.8 |
| 3 | Uric acid | Enzymatic method (uricase) | 3.4 -7 mg/dL | 14.2 |
| 4 | Calcium | Spectrophotometry (Arsenazo III) | 8.1- 10.4 mg/dL | 5.1 |
| 5 | Inorganic phosphorus | Spectrophotometry (Molybdate) | 0.87 -1.45 mmol/L | 14.9 |
| 6 | SGOT | Kinetic Method | 18 -37 U/L | 16.8 |
| 7 | SGPT | Kinetic Method | 22-40 U/L | 17.2 |
| 8 | ALP | Colorimetricmethod (p-nitrophenyl phosphate) | 30 - 120 U/L | 10.1 |
| 9 | Total Protein | Colorimetric method (Biuret) | 6 - 8 g/dL | 4.9 |
| 10 | A/G Ratio | BCG with Biuret | 1 - 2 | 13.1 |
| 11 | Triglycerides | Enzymatic method | 60 - 160 mg/dL | 10.4 |
| 12 | Cholesterol | Enzymatic method | 130 - 230 mg/dL | 5.8 |
| 13 | HDL-C | Immunological method | 30 - 60 mg/dL | 6.8 |
| 14 | LDL-C | Immunological method | 16 - 32 mg/dL | 17.9 |
| 15 | Amylase | Spectrophotometric method | Upto 85 U/L | 4.9 |
| 16 | Glucose | Enzymatic method (GOD- POD) | 75 -115 mg/dL | 22.6 |
| 17 | Sodium | ISE (Combiline) | 135 - 155 meq/L | 10.0 |
| 18 | Potassium | ISE (Combiline) | 3.5 - 5.5 meq/L | 27.3 |

7. $0^0$-3 for 3rd day samples stored at $0^0$ C
8. $0^0$-7 for 7th day samples stored at $0^0$ C
9. $0^0$-15 for 15th day samples stored at $0^0$ C
10. $0^0$-30 for 30th day samples stored at $0^0$ C

The following 18 analytes were studied:

Glucose, urea, creatinine, uric acid, calcium, inorganic phosphorus, SGOT, SGPT, ALP, amylase, total protein, A/G ratio, triglycerides, cholesterol, HDL-C, LDL-C, sodium and potassium.

All assays were performed on the RANDOX (Randox Laboratories Limited, UK), according to the manufacturer's specifications by using proprietary reagents at the Department of Biochemistry.

Intra-assay analytical CV's were determined by two levels of control materials (Randox Laboratories Limited, UK) (N = 10, from each level on the same plate) on the same day before biochemical analysis (TABLE 1). For accuracy and internal quality check, two levels of control materials and the test samples were assayed in the same analytical run at each assay point during the process. All values of quality control samples for each analyte were within ± 2 SD (standard deviation) of their respective target means during the entire procedures.

## DISCUSSION

In this study, effect of storage of serum at room temperature (23±1 °C) and refrigeration (4±1 °C and 0 °C) for 0, 3,7,15,30 days on 18 sera analytes were analysed. Donnelly et al[7] found that the stability of 25 analytes from serum of healthy donors and stored at room temperature and 4 degree °C over 48 h, 14 days and 4 months respectively. All 10 analytes were stable at 2 temperature for specified times.

Bobby et al[8] reported the stability of 24 analytes after immediate separation of serum and stored at room temperature (25 degree °C) and analyzed in 0, 2, 4, 8, 16, 24, 32, 40, 48 and 56 h after collection. All analytes in serum were stable over 56 h periods.

Heins et al[9] studied the effects of storage time and temperature on 22 serum analytes. In serum at +9 degree °C for seven days the mean changes in phosphorus exceeded significantly. In serum at room temperature, phosphorus, uric acid and triacylglycerols increased continuously.

By comparing the above studies, our results for serum analytes were almost consistent with those obtained. By this study we found that of the 18 sera analytes that we measured on 3rd day at room temperature, urea, calcium, SGOT, SGPT, ALP and total proteins did not show significant change quantitatively

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**TABLE 2**

| ANALYTES | 0day | R3 | $4^0$-3 | $0^0$-3 | $4^0$-7 | $0^0$-7 | $4^0$-15 | $0^0$-15 | $4^0$-30 | $0^0$-30 |
|---|---|---|---|---|---|---|---|---|---|---|
| Urea (mg/dl) | 24.4 | 38.7 | 26.1 | 27.2 | 24.5 | 23.5 | 30.4 | 24.4 | 46.8 | 23.6 |
| | (16-39) | (21-103) | (19-40) | (17-43) | (16-34) | (16-30) | (18-68) | (15-33) | (22-216) | (16-40) |
| p value % | | 0.053 58.6 | 0.082 6.96 | 0.167 11.4 | 0.957 0.4 | 0.756 3.6 | 0.060 24.5 | 1.00 0 | 0.014 91.8 | 0.572 3.2 |
| Creatinine (mg/dl) | 0.910 | 0.64 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | (0.7-1) | (0.4-1) | (0.7-1.1) | (0.6-1) | (0.8-1.2) | (0.8-1.2) | (0.6-0.9) | (0.7-1.2) | (0.6-1) | (0.7-1.2) |
| p value % | | 0.006 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 | 0.010 9.8 |
| Uric acid (mg/dl) | 5.00 | 3.37 | 4.80 | 4.70 | 5.10 | 5.30 | 4.00 | 4.70 | 3.30 | 4.60 |
| | (3.4-6.8) | (0-6.6) | (3.6.9) | (3.1-6.5) | (3.2-6.8) | (3.2-7.4) | (1-6.6) | (2.8-6) | (0.8-6.4) | (3-6) |
| p value % | | 0.047 32.6 | 0.168 4.0 | 0.081 6.0 | 0.678 2.0 | 0.081 6.0 | 0.096 20 | 0.081 6.0 | 0.014 34 | 0.037 8.0 |
| Calcium (mg/dl) | 9.68 | 9.72 | 9.9 | 9.10 | 9.00 | 7.90 | 10.10 | 10.00 | 10.10 | 9.10 |
| | (8.1-11.1) | (8.8-11) | (9.3-11.1) | (5.6-10.1) | (7.4-10.9) | (6.9-9.2) | (8-10.9) | (8.8-10.8) | (7.6-11.7) | (8.5-11) |
| p value % | | 0.864 0.41 | 0.492 2.2 | 0.170 5.9 | 0.316 7.0 | 0.000 18.3 | 0.187 4.3 | 0.374 3.3 | 0.336 4.3 | 0.960 0.2 |
| Phosphorus (mg/dl) | 3.29 | 3.79 | 3.6 | 3.6 | 3.2 | 3.1 | 3.5 | 3.5 | 5.2 | 4.00 |
| | (2.4-4.9) | (1.7-5.9) | (2.5-5.2) | (2.6-4.8) | (2.3-4.5) | (2.4-4.8) | (2.2-4) | (2.5-5.7) | (3.1-9.6) | (2.9-7) |
| p value % | | 0.028 15.1 | 0.323 9.4 | 0.242 9.4 | 0.754 2.7 | 0.397 5.8 | 0.431 6.3 | 0.494 6.3 | 0.001 58 | 0.073 21.5 |
| SGOT (U/L) | 22.5 | 36.9 | 22.8 | 23.7 | 27.1 | 23.5 | 31.2 | 22.2 | 22.9 | 19.3 |
| | (16-35) | (17-76) | (15-35) | (16-35) | (19-39) | (15-39) | (18-85) | (12-33) | (19-28) | (14-27) |
| p value % | | 0.086 64 | 0.846 1.3 | 0.489 5.3 | 0.031 20.4 | 0.405 4.4 | 0.239 38.7 | 0.867 1.3 | 0.864 1.7 | 0.014 14.2 |
| SGPT (U/L) | 31.7 | 52.3 | 32.9 | 30.0 | 35.3 | 28.4 | 34.4 | 24.9 | 29.2 | 25.3 |
| | (15-79) | (15-84) | (14-84) | (14-68) | (16-80) | (15-89) | (16-80) | (15-42) | (15-65) | (12-60) |
| p value % | | 0.068 65 | 0.669 3.9 | 0.458 5.3 | 0.032 12.9 | 0.362 10.4 | 0.177 8.5 | 0.155 21.4 | 0.467 7.8 | 0.373 20.1 |
| ALP (U/L) | 87.6 | 81.4 | 83.7 | 92.8 | 97.2 | 88.9 | 94.0 | 92.4 | 119.5 | 86.4 |
| | (46-135) | (15-157) | (42-167) | (43-195) | (52-179) | (71-210) | (47-199) | (44-148) | (23-207) | (44-128) |
| p value % | | 0.608 7.0 | 0.445 4.4 | 0.452 5.9 | 0.041 10.9 | 0.197 12.9 | 0.433 7.3 | 0.234 5.4 | 0.537 36.4 | 0.575 1.3 |
| Protein (g/dL) | 7.230 | 6.97 | 7.00 | 6.7 | 8.00 | 7.8 | 7.4 | 7.2 | 7.2 | 6.6 |
| | (5.9-7.9) | (6.2-7.5) | (6.3-7.6) | (6.2-7.6) | (7.2-8.8) | (7-8.4) | (5.7-8.1) | (6.6-7.9) | (5.5-7.9) | (6-7.4) |
| p value % | | 0.142 3.5 | 0.397 3.1 | 0.026 7.3 | 0.030 10.6 | 0.026 7.9 | 0.421 2.3 | 0.908 0.4 | 0.916 0.4 | 0.012 8.7 |
| A/G Ratio | 1.69 | 1.99 | 2.00 | 2.1 | 1.6 | 1.7 | 1.4 | 1.6 | 2.0 | 1.9 |
| | (1.5-1.8) | (1.4-2.6) | (1.3-2.6) | (1.5-2.8) | (1.2-1.9) | (1.2-1.9) | (1.2-1.9) | (1.2-1.9) | (1.5-1.9) | (1.3-1.8) |
| p value % | | 0.022 17.7 | 0.061 17.8 | 0.003 24.2 | 0.537 5.3 | 0.946 0.6 | 0.097 17.1 | 0.576 5.3 | 0.000 17.8 | 0.031 12.4 |
| TG (mg/dl) | 118.3 | 121.4 | 129.2 | 106.6 | 113.4 | 125.3 | 112.1 | 116.6 | 76.5 | 94.4 |
| | (40-206) | (50-249) | (42-221) | (44-194) | (35-208) | (37-199) | (41-177) | (40-193) | (30-166) | (30-168) |
| p value % | | 0.712 2.6 | 0.159 9.2 | 0.521 9.9 | 0.168 4.1 | 0.142 5.9 | 0.459 5.2 | 0.761 1.4 | 0.003 35.3 | 0.011 20.2 |
| CHOLESTEROL | 171.8 | 163.6 | 162.6 | 160.6 | 174.7 | 168.3 | 152.4 | 159.1 | 160.4 | 155.3 |
| (mg/dl) | (125-207) | (133-203) | (135-201) | (113-193) | (141-230) | (136-209) | (119-200) | (115-208) | (136-211) | (128-208) |
| p value % | | 0.134 1.8 | 0.155 5.3 | 0.089 6.9 | 0.510 1.6 | 0.519 2.0 | 0.005 11.3 | 0.025 7.4 | 0.170 6.6 | 0.038 9.6 |
| HDL (mg/dl) | 42.1 | 31.7 | 41.7 | 41.1 | 41.9 | 42.0 | 39.1 | 39.8 | 36.1 | 39.3 |
| | (25-53) | (11-56) | (28-58) | (27-58) | (28-63) | (26-65) | (21-58) | (27-45) | (26-45) | (31-50) |
| p value % | | 0.010 24.7 | 0.759 0.9 | 0.521 2.3 | 0.928 0.47 | 0.961 0.23 | 0.197 7.1 | 0.111 5.4 | 0.058 14.2 | 0.210 6.6 |
| LDL (mg/dl) | 109.1 | 106.9 | 94.5 | 92.6 | 109.9 | 101.3 | 90.6 | 94.8 | 89.9 | 89.1 |
| | (80-141) | (76-131) | (76-132) | (74-129) | (81-167) | (77-147) | (66-138) | (62-132) | (0-144) | (0-141) |
| p value % | | 0.000 59.4 | 0.003 13.4 | 0.000 15.1 | 0.871 0.7 | 0.055 7.1 | 0.001 16.9 | 0.006 13.1 | 0.166 17.5 | 0.073 18.3 |
| AMYLASE | 85.9 | 79.7 | 80.2 | 78.2 | 92.8 | 90.2 | 84.3 | 87.2 | 89.3 | 86.7 |
| (U/L) | (60-128) | (55-116) | (56-115) | (52-112) | (57-137) | (64-135) | (54-124) | (53-118) | (63-131) | (53-128) |
| p value % | | 0.006 7.2 | 0.027 6.6 | 0.020 8.9 | 0.014 8.0 | 0.046 5.0 | 0.698 1.8 | 0.725 1.5 | 0.543 3.95 | 0.792 0.93 |
| GLUCOSE | 99.8 | 68.7 | 99.1 | 99.3 | 59.8 | 101.6 | 49.2 | 87.5 | 44.6 | 88.6 |
| (mg/dl) | (89-137) | (16-138) | (83-144) | (89-133) | (10-106) | (90-144) | (30-115) | (58-107) | (30-113) | (76-106) |
| p value % | | 0.032 31.2 | 0.793 0.7 | 0.753 0.5 | 0.018 40.0 | 0.370 1.8 | 0.001 50.7 | 0.015 12.3 | 0.000 55.3 | 0.032 11.2 |
| SODIUM | 141.5 | 145.1 | 143.9 | 146.0 | 164.8 | 164.0 | 157.4 | 159.7 | 158.5 | 161.6 |
| (mEq/L) | (132-149) | (130-160) | (128-154) | (136-162) | (154-180) | (149-182) | (140-174) | (141-178) | (152-178) | (141-203) |
| p value % | | 0.223 2.5 | 0.296 1.7 | 0.123 3.2 | 0.000 16.5 | 0.000 15.9 | 0.000 11.2 | 0.001 12.9 | 0.000 12.0 | 0.015 14.2 |
| POTASSIUM | 3.806 | 4.62 | 4.4 | 4.6 | 5.3 | 5.3 | 4.7 | 5.0 | 5.1 | 5.4 |
| (mEq/L) | (3-4.4) | (3.7-5.1) | (3.4-5.2) | (4.4-5.2) | (4.5-6.3) | (4.7-5.8) | (4.4-5.3) | (4.3-6.2) | (4.4-6.4) | (4.5-7.1) |
| p value % | | 0.002 19.7 | 0.038 14 | 0.042 14 | 0.000 37.3 | 0.000 37.3 | 0.004 21.8 | 0.004 29.5 | 0.000 32.1 | 0.001 39.9 |

but creatinine, uric acid, amylase, glucose and potassium showed significantly instability.

Changes in the concentration of Glucose, phosphorus and creatinine clinically significant with increasing storage temperature. Statistically significant changes from the 0.5 h mean were determined using paired 't' test. The significant change limit was applied to find out clinically significant changes in measured analytes. Statistical analysis with mean, p value of each of all 18 analytes at different temperatures can be noted from TABLE 2.

All commercial reference materials for the18 analytes were within ±2 SD of their respective target means during the entire investigation.

## RESULTS

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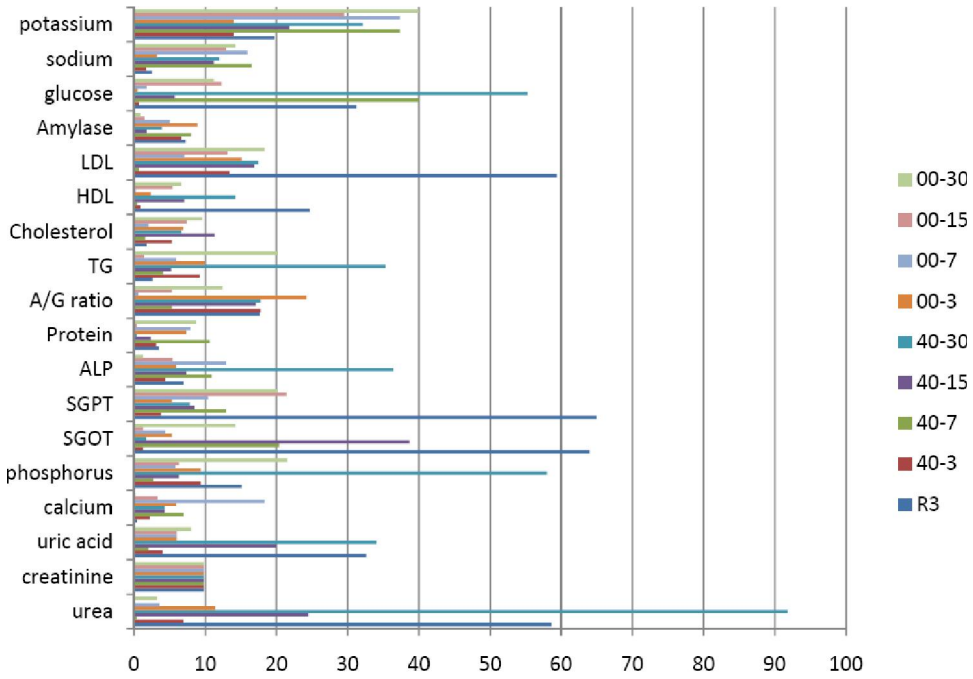


**Figure 1 : Comparison of all analytes with respect to % CV**

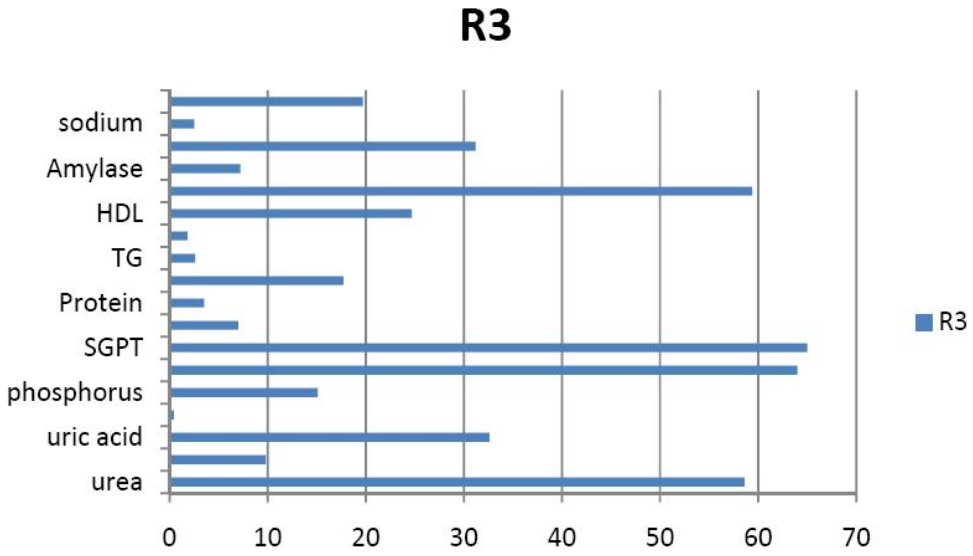


**Figure 2 : Comparison of all analytes at R3 with respect to % CV**

We found that urea showed stability upto 15 days in both storage temperatures (0° C and 4±1° C) and stable upto 30 days only at 0o C. Creatinine was found highly unstable and showed significant p value in all days in both temperatures which was probably due to interference of pseudo-creatinines with the kinetic Jaffe reaction.

Uric acid was stable till 15 days in both temperatures may be due to decreased uric acid solubility in the continuously increasingly acidic environment. Calcium and inorganic phosphorus were stable upto at both temperatures. SGOT was stable till 15 days but showed significant change at 30th day result at both temperatures whereas SGPT and ALP were found that stable till 30th day result but were not stable in at least one of the storage conditions.

Total protein showed significant unstability after 3rd day results at both temperatures. A/G ratio was stable till 15 days then showed variability in both temperatures.

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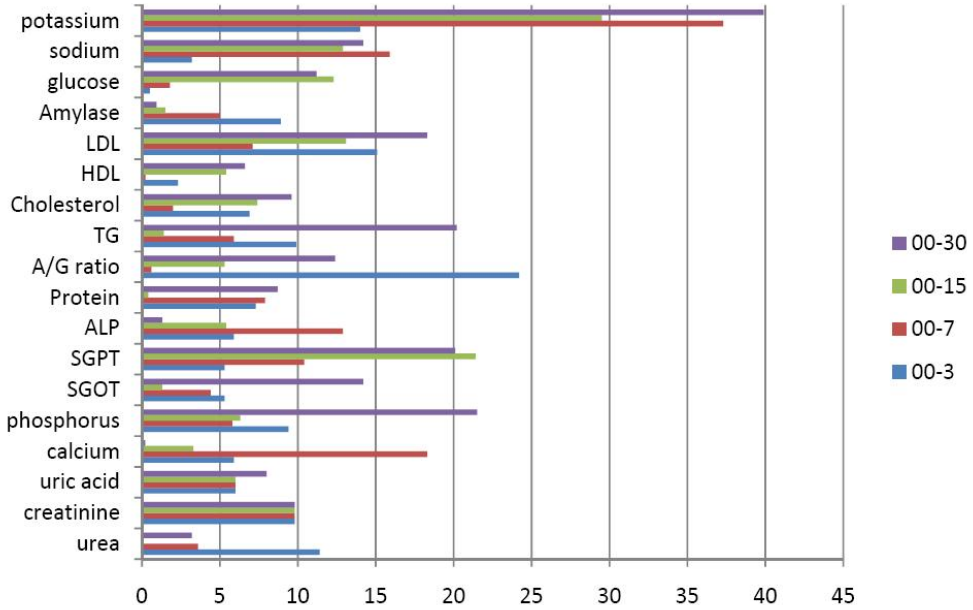


**Figure 3 : Comparison of all analytes at 0° C with respect to % CV**

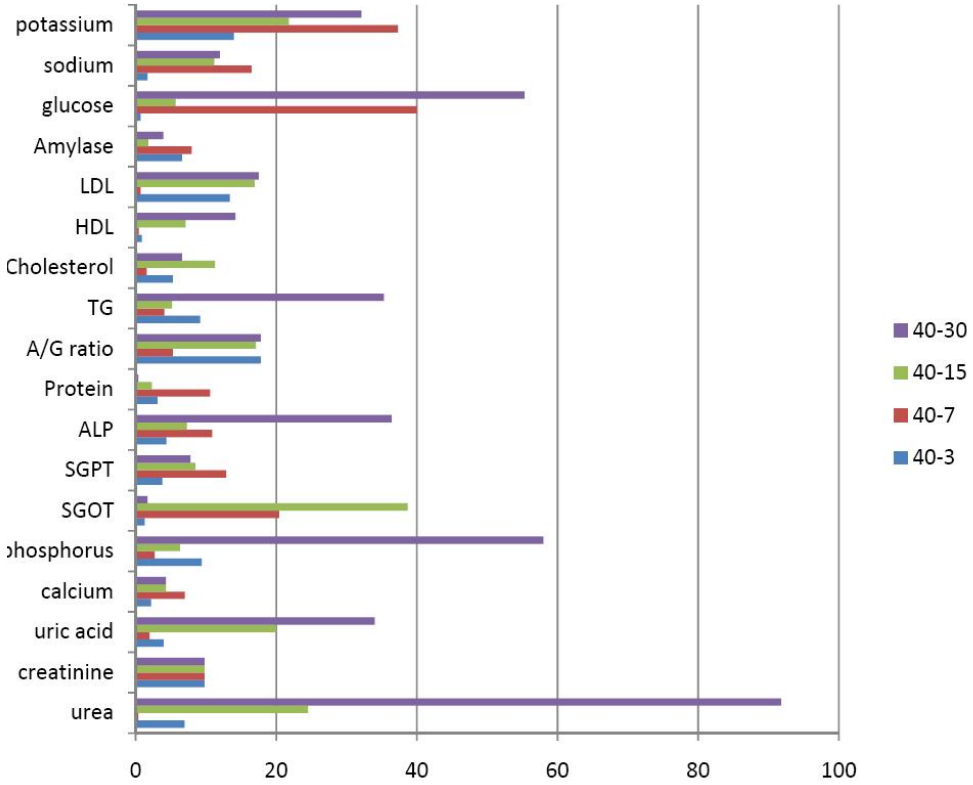


**Figure 4 : Comparison of all analytes at 4° C with respect to % CV**

Amylase and glucose and potassium were highly unstable in all conditions in all days. Triglycerides was found that stable till 15 days at both temperatures but showed significant change at 30th day.

HDL was stable till last day of our study at both temperatures. LDL showed significant variability at various storage conditions.

Statistical analyses were performed by IBM SPSS statistics v20. Beyond this, it is even very useful to check the reliability of technical and instrumental resources that the laboratory will use during the study because molecular alterations of the analytes due to variable stor-

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age conditions can cause misleading results.

## CONCLUSION

In conclusion with this study we hope that the results we have presented can assist to assess which of the analytes may be assayed in serum stored for prolonged times under commonly available storage conditions (refrigerator) when such prolonged storage occurs in advertently or is unavoidable. But we recommend that samples should be analysed in the laboratory within preferably 24 h of collection to ensure valid results with the turn-around time from sample drawing to reporting the analytical result could be shortened.

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